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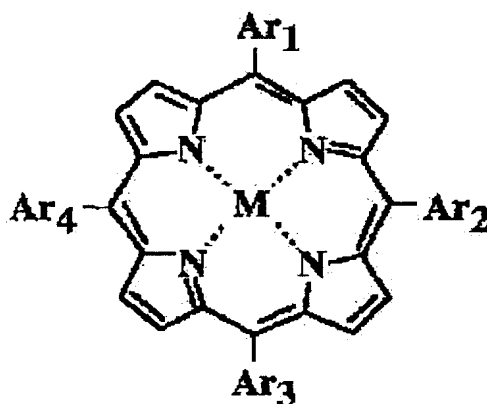
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(54) METAL PORPHYRIN COMPLEX AND PHARMACEUTICAL COMPOSITION CONTAINING THE SAME

(57)Abstract:

PROBLEM TO BE SOLVED: To obtain a new compound having high SOD activity and the character to specifically accumulate on a cancer cell and capable of specifically killing cancer cells by inducing hydroxyl freeradicals through reaction with active oxygen, therefore useful as an anticancer agent with slight side effects.

SOLUTION: This new compound is a compound of the formula [M is a metal atom for forming a complex; Ar1 to Ar4 are each a (substituted) carbocyclic or heterocyclic aromatic group, at least one of them being a cationic group-bearing aromatic group]. The compound of the formula is obtained, for example, by the following process: pyrrole is reacted with an aromatic aldehyde such as benzaldehyde to form a porphyrin ring moiety, which is then cationized with an alkylating agent (e.g. methyl p-toluenesulfonate) such as a halogenated lower alkyl or lower alkyl tosylate and then converted to the corresponding metal complex using a metal or metal compound (e.g. a metal halide such as iron chloride).



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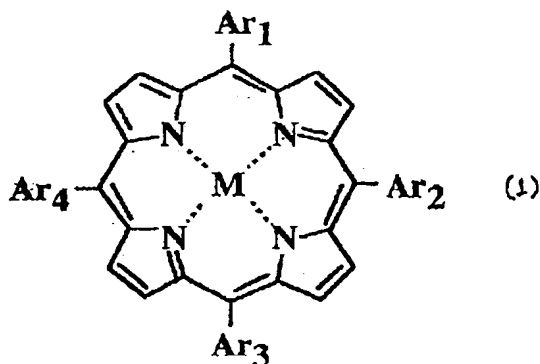
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(54) 【発明の名称】 金属ポルフィリン錯体及びそれを含有してなる医薬組成物

(57) 【要約】 (修正有)

【解決手段】 新規な一般式1のカチオン性金属ポルフィ
リン錯体、およびそれを含有する医薬組成物。

の場でヒドロキシラジカルに変換できるヒドロキシラジ
カル誘発剤及び製薬上許容される担体からなる医薬組成
物に使用できる。



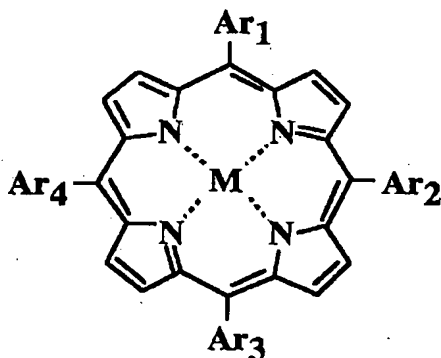
(Mは錯体を形成する金属原子、Ar₁ ~ Ar₄ は独立
して置換基を有してもよい炭素環又は複素環式芳香族基
を示し、そのうち1個以上はカチオン性の基を有する芳
香族基である。)

【効果】 錯体はヒドロキシラジカル誘発剤として有用で
あり、抗癌剤に使用できる。また生体内の活性酸素をそ

【特許請求の範囲】

【請求項1】 一般式(1)

【化1】



(式中、Mは錯体を形成するための金属原子を示し、Ar₁、Ar₂、Ar₃、及び、Ar₄はそれぞれ独立して置換基を有してもよい炭素環式又は複素環式芳香族基を示し、Ar₁、Ar₂、Ar₃、及び、Ar₄の少なくとも1個はカチオン性の基を有する芳香族基である。)で表されるカチオン性金属ポルフィリン錯体。

【請求項2】 Mが、鉄原子、銅原子又はマンガン原子である請求項1に記載のカチオン性金属ポルフィリン錯体。

【請求項3】 Ar₁、Ar₂、Ar₃、及び、Ar₄の少なくともひとつが、N-低級アルキル-4-ピリジル基である請求項1又は2に記載のカチオン性金属ポルフィリン錯体。

【請求項4】 N-低級アルキル-4-ピリジル基が、N-メチル-4-ピリジル基である請求項3に記載のカチオン性金属ポルフィリン錯体。

【請求項5】 Ar₁、Ar₂、Ar₃、及び、Ar₄の少なくともひとつが、4-N, N, N-トリ低級アルキルアミノフェニル基である請求項1又は2に記載のカチオン性金属ポルフィリン錯体。

【請求項6】 4-N, N, N-トリ低級アルキルアミノフェニル基が、4-N, N, N-トリメチルアミノフェニル基である請求項5に記載のカチオン性金属ポルフィリン錯体。

【請求項7】 請求項1～6のいずれかに記載のカチオン性金属ポルフィリン錯体を含有してなる医薬組成物。

【請求項8】 請求項1～6のいずれかに記載のカチオン性金属ポルフィリン錯体を含有してなるヒドロキシラジカル誘発剤。

【請求項9】 請求項1～6のいずれかに記載のカチオン性金属ポルフィリン錯体を含有してなる抗癌剤。

【請求項10】 生体内の活性酸素をヒドロキシラジカルに変換することができるヒドロキシラジカル誘発剤を含有してなる医薬組成物。

【請求項11】 医薬組成物が抗癌剤である請求項10に記載の医薬組成物。

【発明の詳細な説明】

【0001】

【発明の属する技術分野】本発明は、新規なカチオン性金属ポルフィリン錯体、それを含有してなる医薬組成物に関する。より詳細には、本発明の医薬組成物は、SOD活性化剤や抗癌剤として有用なものである。本発明のカチオン性金属ポルフィリン錯体は癌細胞に集積されやすく、副作用の少ない選択的な各種腫瘍の治療剤として有用である。

【0002】

【従来の技術】近年、癌治療の方法として薬剤による化学療法が多く用いられるようになった。しかし、多くの薬剤は、癌細胞のみへの特異性を持たないため癌細胞とともに正常細胞にも作用を及ぼし、このために激しい副作用を起こし、化学療法が必ずしも有効に働いているとは言えないのが現状である。例えば、臨床で用いられている抗癌剤のひとつであるシスプラチンは子宮癌に有効であることが臨床的明かにされているが、有効性と共に多くの副作用も報告されている。また、インターフェロンやTNFやCSFなどの生理活性蛋白質が、癌特異性を有することから注目されていたが、抗癌作用自体が充分でないことや、経口投与できないことなどから抗癌剤として広く使用されるに至っていない。

【0003】ところで、生体内で誘発する活性酸素種は、生体内に侵入した異種生物を死滅させるなど生体の生理活性を維持してゆく上で大きな働きをしているのであるが、同時に必要以上に生体内で生成した活性酸素種は自己の組織をも破壊することがあり、必要以上の活性酸素の生成は、有害であるばかりでなく老化の一因であるともいわれている。この活性酸素種の毒性を利用して、生体内の酸素を活性酸素種に変換して癌細胞を非特異的に攻撃することにより、癌細胞を死滅させる抗癌剤も開発されているが、非特異的であり正常細胞をも攻撃することから多くの副作用を引き起こしている。

【0004】一方、ある種のポルフィリン系化合物が癌細胞などに集積し、これにレーザー光を照射することにより、生体内の酸素を活性化させて活性酸素種を生成させて癌細胞を死滅させることが見出され、比較的特異性の高い抗癌剤のひとつとして使用されてきているが、レーザー光を病巣部に照射させなければならず、病巣部が内部にある場合には効果がないなどの欠点を有するものである。

【0005】

【発明が解決しようとする課題】本発明は、癌細胞に特異的に作用する新規な作用機構に基づく、副作用の少ない抗癌剤を提供するものである。癌細胞では正常細胞に比べて、そのSOD活性が低下しており活性酸素の放出量が増加していることが報告されている(A.V.Peskin, et al., FEBS Lett., 78, 41 (1977); V.Leroyer, et al., Cancer Res., 47, 4771 (1987))。本発明者らは、

この点に着目し、癌細胞が放出する活性酸素を、その場でより高い反応性を有するヒドロキシラジカル($\cdot\text{OH}$)に変換することができれば、癌細胞を特異的に攻撃することができる新しい抗癌メカニズムを構築することができると考えた。

【0006】本発明者らは、新規な金属ポルフィリン錯体が優れたSOD活性を有し、かつ、癌細胞に特異的に集積する性質を有し、活性酸素と反応してヒドロキシラジカルを誘発させ癌細胞を特異的に死滅させることができることを見出した。即ち、本発明は、副作用が少なく安全性の高いSOD活性を有する新規なカチオン性金属ポルフィリン錯体を提供する。また、本発明の新規なカチオン性金属ポルフィリン錯体は、癌細胞に特異的に集積するために、副作用の少ない抗癌剤として有用であり、本発明は新規な抗癌剤を提供するものである。

【0007】

【課題を解決するための手段】本発明は、次の一般式(1)

【0008】

【化2】

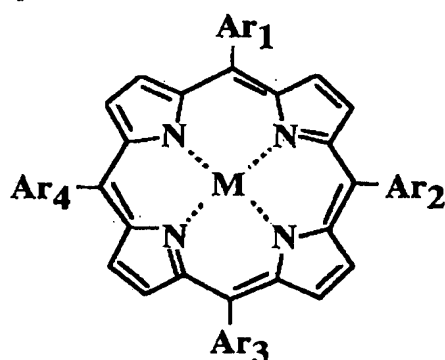


表1 癌細胞と正常細胞におけるSOD活性の比較

細 胞	I C ₅₀ [$\times 10^3$ U/mg蛋白質]
LLC-WRC-256細胞	8 \pm 0.6
BRL-3A細胞	22 \pm 1.2
Cu, Zn-SOD	760 \pm 45

【0012】このように癌細胞においては、正常細胞に比べてSOD活性が大きく低減している。本発明の前記一般式(1)で表されるカチオン性金属ポルフィリン錯体は、SOD活性を有しており、生体内でスーパーオキシドラジカルと特異的に反応し過酸化水素を生成する。さらに、本発明のカチオン性金属ポルフィリン錯体は、中心に金属を有しているために、生成した過酸化水素がハーバー・バイス型反応を起こし、極めて毒性の高いヒドロキシラジカルを生成し、癌細胞のみを特異的に攻撃する。

【0013】一方、生体内の抗酸化酵素が機能している正常細胞の場合には、スーパーオキシドラジカルがほと

(式中、Mは錯体を形成するための金属原子を示し、Ar₁、Ar₂、Ar₃、及び、Ar₄はそれぞれ独立して置換基を有してもよい炭素環式又は複素環式芳香族基を示し、Ar₁、Ar₂、Ar₃、及び、Ar₄の少なくとも1個はカチオン性の基を有する芳香族基である。)で表されるカチオン性金属ポルフィリン錯体に関する。

【0009】また、本発明は、前記一般式(1)で表されるカチオン性金属ポルフィリン錯体及び製薬上許容される担体からなる医薬組成物に関する。本発明の医薬組成物は、ヒドロキシラジカル誘発剤であるばかりでなく、抗癌剤として使用することができる。さらに、本発明は生体内の活性酸素を、その場でヒドロキシラジカルに変換することができるヒドロキシラジカル誘発剤及び製薬上許容される担体からなる医薬組成物、特に抗癌剤に関する。

【0010】癌細胞は、正常細胞にくらべ抗酸化酵素(SOD、カタラーゼ等)が欠落していることが知られており、このために癌細胞は正常細胞に比べてスーパーオキシドラジカルを多量に発生している。例えば、癌細胞のLLC-WRC-256細胞と、正常細胞のBRL-3A細胞、及び、天然の抗酸化酵素(Cu, Zn-SOD)のSOD活性を測定すると次の表1ようになる。

【0011】

【表1】

んど発生しておらず、正常細胞においては本発明のカチオン性金属ポルフィリン錯体が仮に存在していても、スーパーオキシドラジカルと反応することができずヒドロキシラジカルを生成することができない。したがって、本発明のカチオン性金属ポルフィリン錯体は、選択的かつ優れたSOD活性を持つヒドロキシラジカル誘発剤であるため副作用の少ない癌細胞特異性の高い新しい抗癌剤として極めて有用なものである。

【0014】本発明のカチオン性金属ポルフィリン錯体は、ポルフィン骨格に4個の芳香族基を有するものであり、かつ、4個の芳香族基のうちの少なくとも1個にカチオン性の基を有していることを特徴とするものであ

る。4個の芳香族基は、それぞれ独立して、炭素環式のものであっても複素環式のものであってもよく、単環式のもでも多環式のものであってもよい。芳香族基としては、炭素環式のものとしては例えば、ベンゼン環、ナフタレン環などから誘導される基であり、複素環式のものとしては、1個又は2個以上の窒素原子、酸素原子又は硫黄原子を有する5～10員の単環式又は縮合環式の複素環から誘導される基であり、例えば、ビリジン環、ピリミジン環、アゾール環などから誘導される基である。好ましい芳香族基としては、フェニル基や4-ピリジル基などが挙げられる。

【0015】これらの芳香族基はSOD活性や抗癌作用に悪影響を与えない置換基を有していてもよい。芳香族基における置換基としては、炭素数1～10、好ましくは1～6の直鎖又は分枝状の低級アルキル基、アミノ基、前記した低級アルキル基で置換されているアミノ基、前記した低級アルキル基からなる低級アルコキシ基などが挙げられる。

【0016】芳香族基が有するカチオン性の基としては、アンモニウム基やスルホニウム基などが挙げられるが、第四級アンモニウム基が好ましい。本発明のカチオン性の基は芳香族基の置換基として有していてもよいが、芳香族基の異種原子がカチオン化されたものであってもよい。カチオン性の基を有する芳香族基としては、例えば、4-N, N, N-トリメチルアミノフェニル基、4-N, N, N-トリエチルアミノフェニル基などの4-N, N, N-トリ低級アルキルアミノフェニル基、N-メチル-4-ピリジル基、N-エチル-4-ピリジル基などのN-低級アルキル-4-ピリジル基などが挙げられる。

【0017】本発明の前記一般式(1)の Ar_1 、 Ar_2 、 Ar_3 、及び、 Ar_4 の少なくとも1個はカチオン性の基を有する芳香族基であるが、好ましくはこれらの芳香族基のうちの2個以上がカチオン性の基を有するものである。本発明の前記一般式(1)で表されるカチオン性金属ポルフィリン錯体のポルフィリン環部分のものとしては、 Ar_1 、 Ar_2 、 Ar_3 、及び、 Ar_4 の全てが、N-メチル-4-ピリジル基などのN-低級アルキル-4-ピリジル基、又は、4-N, N, N-トリメチルアミノフェニル基などの4-N, N, N-トリ低級アルキルアミノフェニル基である化合物、 Ar_1 、 Ar_2 、及び、 Ar_3 がN-メチル-4-ピリジル基などのN-低級アルキル-4-ピリジル基、又は、4-N, N, N-トリメチルアミノフェニル基などの4-N, N, N-トリ低級アルキルアミノフェニル基であり、 Ar_4 がフェニル基である化合物、 Ar_1 、及び、 Ar_2 がN-メチル-4-ピリジル基などのN-低級アルキル-4-ピリジル基、又は、4-N, N, N-トリメチルアミノフェニル基などの4-N, N, N-トリ低級アルキルアミノフェニル基であり、 Ar_3 、及び、 Ar_4 が

フェニル基である化合物、 Ar_1 、及び、 Ar_3 がN-メチル-4-ピリジル基などのN-低級アルキル-4-ピリジル基、又は、4-N, N, N-トリメチルアミノフェニル基などの4-N, N, N-トリ低級アルキルアミノフェニル基であり、 Ar_2 及び Ar_4 がフェニル基である化合物などが挙げられる。

【0018】本発明の前記一般式(1)における中心金属Mとしては、SOD活性や抗癌作用を示すものであれば特に制限はないが、好ましくは、鉄原子、銅原子又はマンガン原子などが挙げられる。

【0019】本発明の前記一般式(1)で表されるカチオン性金属ポルフィリン錯体は、公知の方法に従って製造することができる。例えば、ピロールと芳香族アルデヒドとを反応させてポルフィリン環部分を製造し、これをハロゲン化低級アルキルや低級アルキルトシレートなどのアルキル化剤でカチオン化し、次いで金属又は金属化合物、例えば金属ハロゲン化物などを用いて金属錯体とする方法により製造することができる。

【0020】本発明者らは、癌細胞としてウォーカーラット(Walker rat)癌由来のLLC・WRC・256細胞及びSOD活性の異なる数種の癌細胞を用いて、各種の金属ポルフィリン錯体の抗癌作用を検討した。この試験の手順を模式的に図1に示す。なお、試験を行った化合物とその略称は次の通りである。

【0021】FeTM4PyP：一般式(1)の Ar_1 、 Ar_2 、 Ar_3 、及び、 Ar_4 が、N-メチル-4-ピリジル基である鉄錯体。

MnTM4PyP：一般式(1)の Ar_1 、 Ar_2 、 Ar_3 、及び、 Ar_4 が、N-メチル-4-ピリジル基であるマンガン錯体。

CuTM4PyP：一般式(1)の Ar_1 、 Ar_2 、 Ar_3 、及び、 Ar_4 が、N-メチル-4-ピリジル基である銅錯体。

FeTMAP：一般式(1)の Ar_1 、 Ar_2 、 Ar_3 、及び、 Ar_4 が、4-N, N, N-トリメチルアミノフェニル基である鉄錯体。

FeTSPP：一般式(1)の Ar_1 、 Ar_2 、 Ar_3 、及び、 Ar_4 が、4-スルホネートフェニル基である鉄錯体。

【0022】ポルフィリン溶液の調製は培養液を用いて行い、ポルフィリン濃度が最終的に10、50、100 $\mu\text{g}/\text{ml}$ になるように調整した。測定は12穴プレートに展開した癌細胞に金属ポルフィリン錯体溶液を添加し培養し、添加3日後にトリパンブルー染色法により各癌細胞の細胞生存率を測定し抗癌効果を評価した。

【0023】また、これらの金属ポルフィリン錯体のSOD活性を、T. Ohse, et al., Porphyrins, 6, 137 (1997)に記載されている方法に準じて、ストップフロー法による活性酸素($\text{O}_2 \cdot$)の不均化速度定数(k_{cat})により評価した。 k_{cat} はポルフィリン錯体

のHEPES/HEPES・Na緩衝液(pH8.1)と KO_2 のDMSO溶液とを36℃で反応させ、 O_2^- の極大吸収波長である245nmの吸光度の減衰から求めた。金属ポルフィリン錯体及び抗酸化酵素における k_{cat} 及び IC_{50} を次の表2に示す。なお、 k

k_{cat} の値が大きいほど、また、 IC_{50} の値が小さいほどSOD活性は高い。

【0024】

【表2】

表2 各種ポルフィリン錯体の k_{cat} 値と IC_{50}

試験化合物	k_{cat} [$\times 10^{-6} \text{M}^{-1} \text{s}^{-1}$]	IC_{50} [$\mu\text{g}/\text{ml}$]
Cu, Zn-SOD	2310	0.3
FeTM4PyP	22	0.8
FeTM4PyMPP	5.4	1.6
FeDM4PyDPP	3.8	1.8
MnTM4PyP	22	0.7
FeTSPP	---	---

【0025】さらに、癌細胞が有する細胞内SOD活性は細胞のホモジネート溶液を調製し、CLAを用いた化学発光法により算出した。そして、発生したラジカル種($\cdot\text{OH}$)は、スピントラップ剤としてDMPOを用いたESRにより測定した。

【0026】図2に、LLC・WRC・256細胞による、発明のカチオン性金属ポルフィリン錯体($100 \mu\text{g}/\text{ml}$)及びウシ赤血球由来の抗酸化酵素($50 \mu\text{g}/\text{ml}$)を用いた癌細胞の死滅率を示す。LLC・WRC・256細胞のトリパンブルー染色法による細胞生存率評価の結果、抗酸化酵素はSOD活性は有しているがそれ自体は抗癌効果が極めて低いものである。また、金属の比較ではSOD活性および $\cdot\text{OH}$ 産生能の高いFeTM4PyPが最も高い抗癌効果を示した。優れたSOD活性を有するMn錯体はあまり効果が認められなかったのはMn錯体の低い $\cdot\text{OH}$ 産生能によるものと考えられる。一方、Fe錯体同様優れた $\cdot\text{OH}$ 産生能を示すCu錯体では、SOD活性が低いため、Fe錯体に比べて抗癌効果は低いものとなったと考えられる。

【0027】また、前記の結果をSOD活性の指標となる活性酸素(O_2^-)の不均化速度定数(k_{cat})の値と共に図3に示す。図3中の数値は、 $10^6 \times k_{\text{cat}}$ ($\text{M}^{-1} \text{s}^{-1}$)であり、因みに抗酸化酵素の値は 2300×10^6 ($\text{M}^{-1} \text{s}^{-1}$)であった。

【0028】次に本発明者らは、カチオン性金属ポルフィリン錯体の癌細胞への集積能について検討した。脂質膜二重層からなる細胞膜との親和性や透過性を考察するために、親水性、疎水性の異なる次の化合物について試験した。

【0029】FeTM4PyP:一般式(1)の Ar_1 、 Ar_2 、 Ar_3 、及び、 Ar_4 が、N-メチル-4-ピリジル基である鉄錯体。

FeTM4PyMPPP:一般式(1)の Ar_1 、 Ar_2 、及び Ar_3 が、N-メチル-4-ピリジル基であり、 Ar_4 がフェニル基である鉄錯体。

Fe cis -DM4PyDPP:一般式(1)の Ar_1 、及び Ar_2 が、N-メチル-4-ピリジル基であり、 Ar_3 、及び Ar_4 がフェニル基である鉄錯体。

Fe trans -DM4PyDPP:一般式(1)の Ar_1 、及び Ar_3 が、N-メチル-4-ピリジル基であり、 Ar_2 、及び Ar_4 がフェニル基である鉄錯体。

【0030】癌細胞への集積挙動は蛍光顕微鏡を用いてポルフィリン錯体の赤色蛍光を観察することにより確認した。さらに原子吸光分析より細胞内のポルフィリン錯体を定量した。結果を次の表3に示す。

【0031】

【表3】

表3 各種ポルフィリン錯体の癌細胞への集積能

試験化合物	取り込み量 [$\text{fmol}/\text{細胞}$]
FeTM4PyP	1.4 ± 0.1
FeTM4PyMPP	1.9 ± 0.2
FeDM4PyDPP	11 ± 0.8
MnTM4PyP	1.4 ± 0.2
FeTSPP	1.0 ± 0.1

【0032】この結果、疎水性の強いポルフィリン錯体ほど集積能が高いことが確認された。特にFeDM4P

yDPPは短時間のうちに多くの錯体が癌細胞に取り込まれた。ポルフィリン錯体に疎水性基が導入されること

により、疎水的な細胞膜との相互作用が強くなり集積能が増大したと考えられる。

【0033】また、これらの化合物（ $100\mu\text{g}/\text{ml}$ ）についての抗癌効果の経時的な変化を測定した。この結果を図4に示す。図4中の黒三角印はFeTM4PyPを、黒丸印はFeTM4PyMPPを、黒四角印はFeDM4PyDPPをそれぞれ示している。使用した癌細胞は、ウォーカーラット（Walker rat）癌由来のLLC・WRC・256細胞であり、各化合物を $100\mu\text{g}/\text{ml}$ の濃度で使用した。図4の縦軸は癌細胞の生存率を示しており、100%は全癌細胞が生存している状態を示している。

【0034】メソ位にフェニル基2個を有するFeDM4PyDPPは、添加後24時間以内ではほぼ全ての癌細胞を死滅させた。抗癌効果は、FeDM4PyDPP>FeTM4PyP>FeTM4PyMPPの順で減少した。

【0035】LD₅₀（Median Lethal Dose：細胞を50%死滅させるのに必要な薬剤の量）の値を測定した結果を表4に示す。表4に、併せて前述した方法で測定したSOD活性についての k_{cat} の値を示す。

【0036】

【表4】

表4 各種ポルフィリン錯体の k_{cat} 値とLD ₅₀		
試験化合物	k_{cat} [$\times 10^{-6}\text{M}^{-1}\text{s}^{-1}$]	LD ₅₀ [$\mu\text{M}/\text{ml}$]
FeTM4PyP	22	46
FeTM4PyMPP	5.4	150
FeDM4PyDPP	3.8	24
FeTM4PyP	22	--
FeTSPP	--	--

【0037】不均化速度定数（ k_{cat} ）は、カチオン性置換基の減少と共に低下し、FeTM4PyP>FeTM4PyMPP>FeDM4PyDPPの順となった。しかし、FeDM4PyDPPは低いながらもSOD活性を有しており高い集積能が優れた抗癌作用を誘発させた。つまり、FeDM4PyDPPの疎水性により癌細胞への集積が著しく増大することによりカチオン性置換基の減少によるSOD活性の低下を補い、優れた抗癌作用を示したと考えられる。以上のことから、癌細胞への集積能はカチオン性金属ポルフィリン錯体が示す抗癌効果において重要な因子であることがわかった。

【0038】次に本発明の化合物の正常細胞に対する作用を検討した。まず、本発明の化合物及び公知の抗癌抗生物質であるマイトマイシンCを用いて、正常細胞（BRL-3A）の生育実験を行った。結果を図5に示す。図5中の黒丸印はコントロールを示し、黒四角印はFeTM4PyPを添加した場合を示し、黒三角印はMnTM4PyPを添加した場合を示し、白丸印はマイトマイシンCを添加した場合を示す。従来の抗癌剤であるマイトマイシンCを添加した場合には、時間の経過と共に細胞数が減少し、約70時間後には0になる。一方、本発明の化合物の場合にはほぼコントロールと同様に細胞の増殖が行われている。即ち、本発明の化合物は正常細胞にはほとんど影響を与えないことがわかる。

【0039】さらに、本発明の化合物であるFeTM4PyPを種々の濃度で、正常細胞（BRL-3A細胞）と癌細胞（Walker 256細胞）の培養液中に添加して、各細胞の生存率を測定した。結果を図6に示す。

図6中の白丸印は癌細胞の生存率を示し、黒丸印は正常細胞を示す。癌細胞では、FeTM4PyPの濃度が $50\mu\text{g}/\text{ml}$ でその生存率が約50%程度に、さらに濃度が $100\mu\text{g}/\text{ml}$ では約40%程度に低下するのに対して、正常細胞においては、濃度を上げていってもその生存率はほとんど低下せず、本発明の化合物が正常細胞に対してはほとんど影響を与えないことがわかる。

【0040】以上の結果から、本発明の有効成分は、多くの活性酸素を有する癌細胞において特異的にヒドロキシラジカルを発生させることにより抗癌効果を奏するものであることが判明した。このようなメカニズムによる抗癌剤は、本発明者らによる新規な着想に基づくものであり、本発明は新規なメカニズムによる癌細胞に特異的な新規な抗癌剤を提供するものである。また、本発明の医薬組成物は、経口又は非経口により投与することができ、その有効投与量は、病態や患者により相違するが、一般的には $1\mu\text{g}\sim 1\text{g}$ を1日数回に分けて投与するか連続的に投与する。本発明の医薬組成物は公知の方法により、製剤化することができ、投与方法や患者により適宜製剤することができる。

【0041】

【実施例】次に本発明を実施例に基づいて具体的に説明するが、本発明はこれらの実施例に限定されるものではない。

【0042】実施例1（ポリフリン環の合成）

三つ口フラスコにあらかじめ Na_2SO_4 で脱水しておいたプロピオン酸300mlを入れ、窒素下、 110°C でベンズアルデヒド7.5ml（0.0707mol）、

ピリジン-4-アルデヒド 12.5 ml (0.1165 mol) を入れ、攪拌、遮光した。次いで、ピロール 12.5 ml (0.1863 mol) をゆっくり滴下した。2時間反応させた後、溶媒を留去した。アンモニア水で中和し、また溶媒を留去した。100℃で減圧乾燥を行った。メタノールで洗浄・過し、残渣を減圧乾燥した。フラッシュカラム（固定相：シリカゲル、展開溶媒：塩化メチレン 100% → メタノール 5% / 塩化メチレン 95%）でポルフィリン（TPP、MPyTPP、DPyDPP、TPyMPP、TPyP）を分離精製した。今回は DPyDPP の *cis* 体、*trans* 体を分離することはできなかった。各ポルフィリンの単離は NMR で確認した。収率は約 2% だった。

【0043】実施例 2（ポルフィリンのメチル化（メソ位のピリジル基の N 原子の四級化））

三つ口フラスコにエタノール 10% (3 ml) / クロロホルム 90% (27 ml) の溶媒を入れ、各ポルフィリン（MPyTPP の場合 0.077 g ($1.3 \times 10^{-4} \text{ mol}$)、DPyDPP の場合 0.235 g ($3.8 \times 10^{-4} \text{ mol}$)、TPyMPP の場合 0.251

$^1\text{H-NMR}$ [270 MHz, DMSO- d_6] : δ

DMPyDPP	9.48 (8H, 2, 6-ピリジル)
	9.20 (8H, ピロール- β)
	9.00 (8H, 3, 5-ピリジル)
	4.73 (12H, N-メチル)
	-3.10 (2H, 内部ピロール)
TPMyMPP	9.71-7.30 (46H)
	4.96 (9H, N-メチル)
	-2.74 (2H, 内部ピロール)
TPMyP	9.44-7.07 (40H)
	4.71 (6H, N-メチル)
	-2.90 (2H, 内部ピロール)

【0046】実施例 3（ポルフィリンへの金属 (Fe) 導入）

三つ口フラスコにコハク酸緩衝液 (pH 4.05) を 150 ml 入れ、80℃、窒素下で、各ポルフィリン（DMPyDPP の場合は 0.257 g ($2.7 \times 10^{-4} \text{ mol}$)、TPMyMPP の場合は 0.207 g ($1.8 \times 10^{-4} \text{ mol}$)）を加え、塩化鉄 (FeCl_3) 4 水和物をポルフィリンの約 10 倍モル量（DMPyDPP に対して 0.425 g ($2.1 \times 10^{-3} \text{ mol}$)、TPMyMPP に対して 0.382 g ($1.9 \times 10^{-3} \text{ mol}$)）入れ、1時間ごとに UV-Vis スペクトルを測定し、ピークのシフト、吸光度から金属導入が確認できるまで反応させた。溶媒を留去後、カラム（固定相：イオン交換樹脂 HP20、展開溶媒：水 → 水 10% / メタノール 90%）で遊離した鉄を分離し、溶媒を留去後減圧乾燥を行い FeDMPyDPP、FeTPMyMPP を得た。収率は約 70% だった。

【0047】実施例 4（細胞培養）

g ($3.9 \times 10^{-4} \text{ mol}$) を加えた。メチル化する N 原子の数の約 4 倍モル量の p-トルエンスルホン酸メチル (MPyTPP に対しては 0.120 ml ($6.4 \times 10^{-4} \text{ mol}$)、DPyDPP に対しては 0.57 ml ($3.1 \times 10^{-3} \text{ mol}$)、TPyMPP に対しては 0.88 ml ($4.7 \times 10^{-3} \text{ mol}$)) を加え、35℃、窒素下で遮光して終夜反応させた。溶媒を留去後、減圧乾燥を行い MDPyPPP (MPyTPP をメチル化したもの)、DPyDPP (DPyPPP をメチル化したもの)、TPMyMPP (TPyMPP をメチル化したもの) を得た。

【0044】TPMyMPP はこの後微量のメタノールに溶かしてジエチルエーテル中で再沈させ、過剰残渣を回収した。MDPyPPP の方は非常に難溶なので、この過程は行わなかった。十分に減圧乾燥を行った。また MDPyTPP は水に不溶であったので、ここでは使用することができなかった。各メチル化の確認は NMR で行った（図 7、図 8 及び図 9 参照）。収率は約 45% だった。

【0045】

凍結保存しておいた細胞を 37℃ の温水で解凍した後、遠沈管に移し、10 分間、1000 回転で遠心分離し、上澄みを捨てて凍結保存の際に用いる DMSO を除き、培養液（以下、MEM と表す。）を入れ懸濁後、培養フラスコに移し培養器内（37℃、炭酸ガス下）で培養した。2-3 日おきに MEM を交換し、十分に細胞が増殖した後、トリプシン処理して細胞をフラスコからはがし遠沈管に移し、10 分間、1000 回転で遠心分離後上澄みを捨て MEM を加え懸濁後、いくつかのフラスコに移し（継代）、細胞を増殖させた。この際、LLC-WRC-256 細胞は Eagle's essential medium（以下、E-MEM と表す。）、BRLE-3A 細胞は Ham's F-12K（以下、F-12K と表す。）を用いて培養した。

【0048】実施例 5（制癌活性評価）

継代培養した細胞をトリプシン処理で培養フラスコよりはがし、遠沈管に移し、10 分間、1000 回転で遠心分離後、上澄みは捨て MEM を加え懸濁後、12 穴のデ

イッシュに1穴につき1mlずつ加えた。1日培養器で培養して細胞を着床させてから、終濃度が10-100 $\mu\text{g}/\text{ml}$ となるように調製した各ポルフィリン錯体及びマイトマイシンC、又はカルボプラチンを添加し、培養器に入れた。評価は一定時間経過後(0.5~120時間後)に培養器から取り出し、トリパンブルー溶液で死滅細胞を染色することで行った。一定時間経過後、MEMを吸引し、PBSで洗浄後、トリパンブルー溶液を入れ、15分間培養器に入れた。吸引後PBSで洗浄し、再びPBSを入れ、培養顕微鏡(OLYMPUS 倒立型培養顕微鏡IMT-2)で観察し、写真撮影(OLYMPUS 顕微鏡写真自動露出撮影装置model PM 10 A)を行った。得られた写真からトリパンブルーで染色された死滅細胞と染まっていない生細胞の数を計測し、細胞生存率を算出した。以上の手順を図1に模式的に示す。

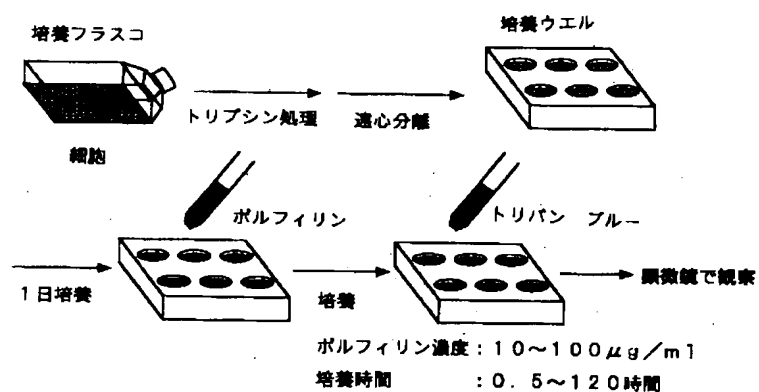
【0049】

【発明の効果】本発明は、癌細胞に多量に存在する活性酸素をヒドロキシラジカル($\cdot\text{OH}$)に変換することにより癌細胞を特異的に死滅させるという新規なメカニズムによる癌の治療方法及びこの方法による新規な抗癌剤を提供するものである。本発明の抗癌剤は、正常細胞に対する副作用が少なく、安全で且つ治療効果の高いものである。また、本発明は、癌細胞の増殖抑制および抗癌作用を有する新規な金属ポルフィリン錯体を提供する。

【図面の簡単な説明】

【図1】図1は、癌細胞の生存率を測定方法を模式的に

【図1】



示したものである。

【図2】図2は、本発明のカチオン性金属ポルフィリン錯体及び抗酸化酵素の癌細胞に対する死滅率をグラフ化して示したものである。

【図3】図3は、各種金属ポルフィリン錯体及び抗酸化酵素の癌細胞に対する死滅率をグラフ化し、そのSOD活性の k_{cat} 値を示したものである。

【図4】図4は、本発明のカチオン性金属ポルフィリン錯体の癌細胞に対する生存率をグラフ化して示したものである。図4中の黒三角印はFeTM4PyPを、黒丸印はFeTM4PyMPPを、黒四角印はFeDM4PyDPPをそれぞれ示している。

【図5】図5は、本発明のカチオン性金属ポルフィリン錯体及び公知の抗癌剤における正常細胞に対する作用をグラフ化して示したものである。

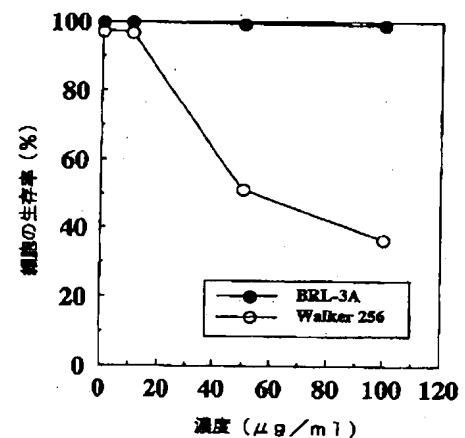
【図6】図6は、本発明のカチオン性金属ポルフィリン錯体による癌細胞及び正常細胞への影響をグラフ化して示したものである。

【図7】図7は、本発明のカチオン性金属ポルフィリン錯体(DMPyDPP)の金属化前の化合物のNMRチャートを示したものである。

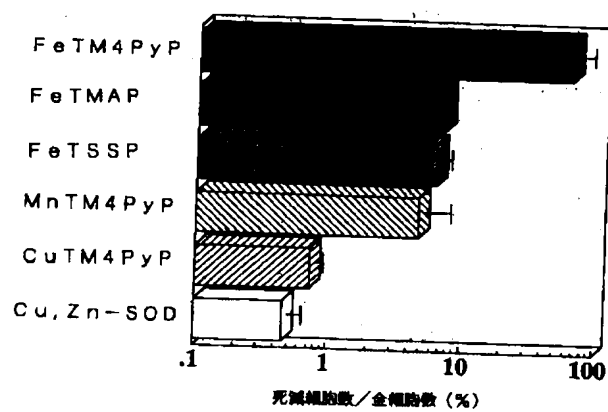
【図8】図8は、本発明のカチオン性金属ポルフィリン錯体(TMPyMPP)の金属化前の化合物のNMRチャートを示したものである。

【図9】図9は、本発明のカチオン性金属ポルフィリン錯体(TMPyP)の金属化前の化合物のNMRチャートを示したものである。

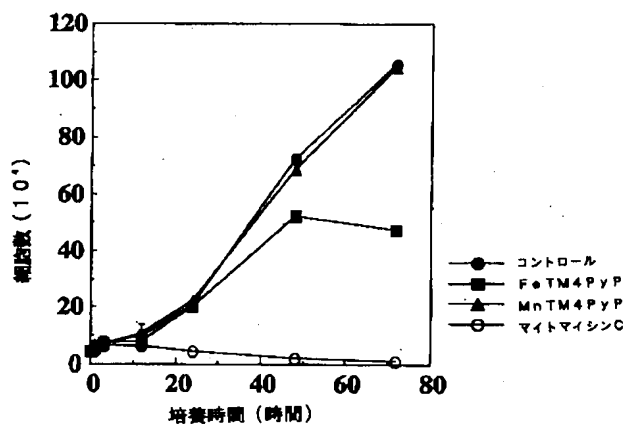
【図6】



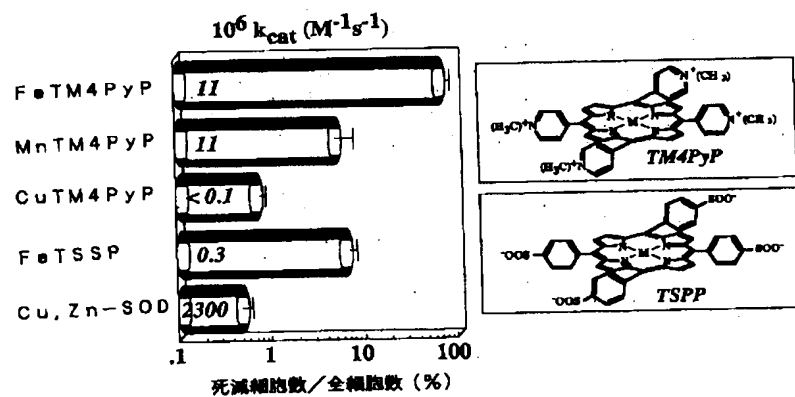
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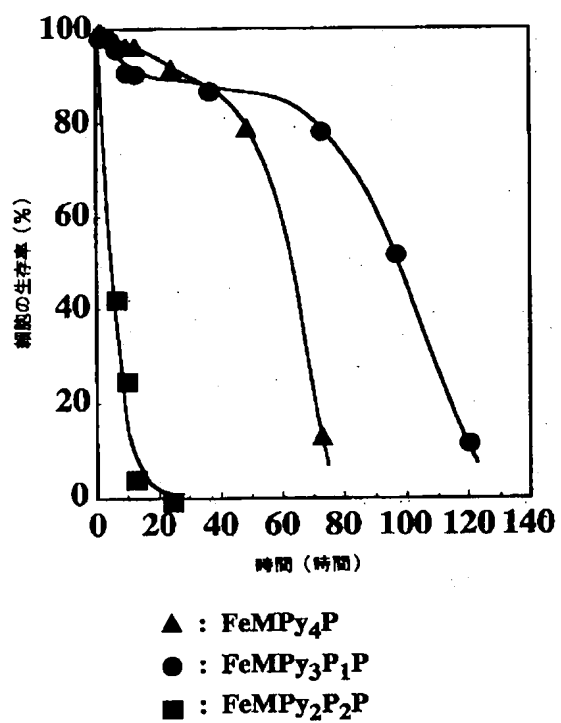
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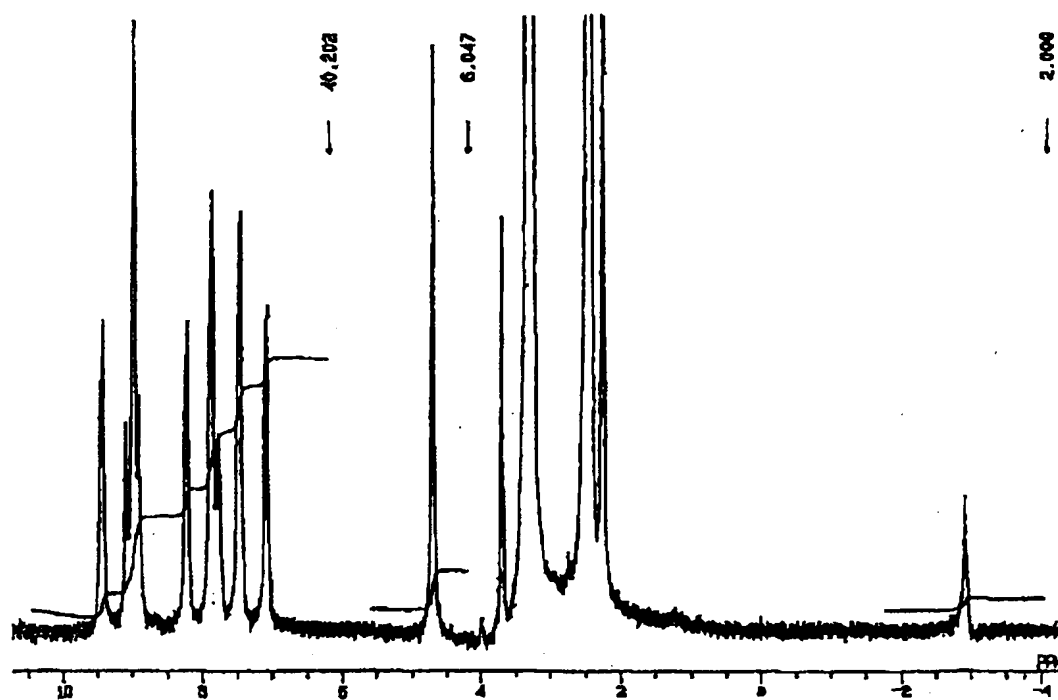
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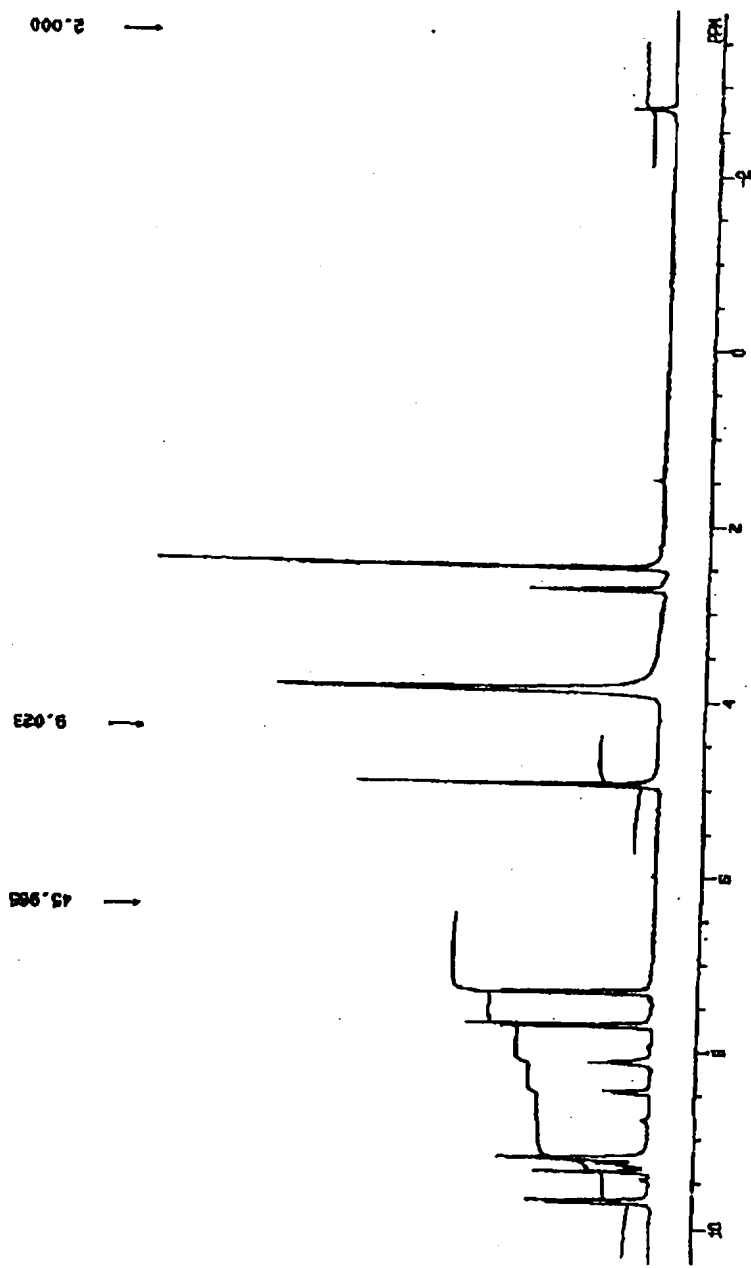
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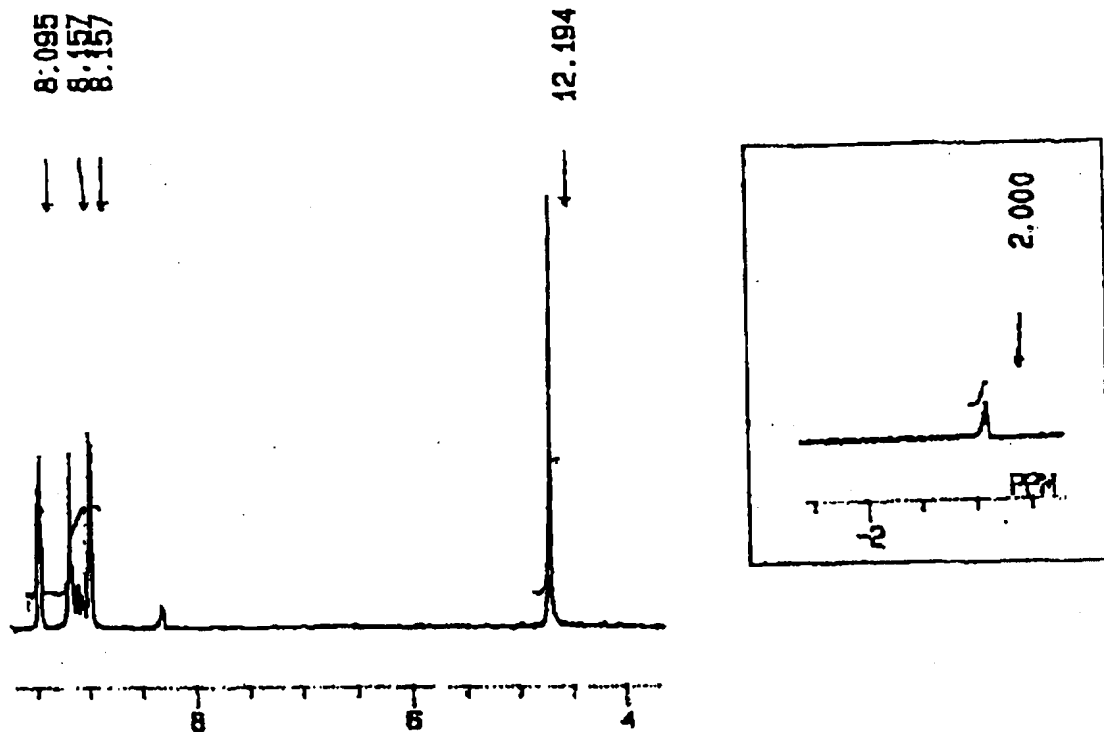
【図7】



【図8】



【図9】



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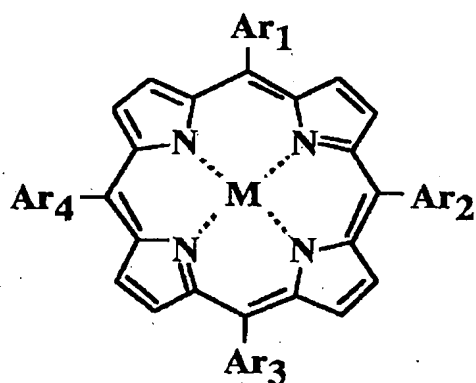
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CLAIMS

[Claim(s)]

[Claim 1]A general formula (1)

[Formula 1]



(Among a formula, M shows the metal atom for forming a complex, Ar₁ it, and) Ar₂, Ar₃, and Ar₄ show carbocyclic or the heterocyclic aromatic group which may have a substituent independently, respectively, At least one piece is an aromatic group which has a cationic basis of Ar₁, Ar₂, Ar₃, and Ar₄. Cationic metalloporphyrin complex expressed.

[Claim 2]The cationic metalloporphyrin complex according to claim 1 whose M is an iron atom, a copper atom, or manganese atoms.

[Claim 3]Ar₁, Ar₂, Ar₃, and the cationic metalloporphyrin complex of Ar₄ according to claim 1 or 2 whose one is an N-low-grade alkyl 4-pyridyl group at least.

[Claim 4]The cationic metalloporphyrin complex according to claim 3 whose N-low-grade alkyl 4-pyridyl group is an N-methyl-4-pyridyl group.

[Claim 5]Ar₁, Ar₂, Ar₃, and the cationic metalloporphyrin complex of Ar₄ according to claim 1 or 2 whose one is 4-N, N, and N-Tori low-grade alkylamino phenyl group at least.

[Claim 6]The cationic metalloporphyrin complex according to claim 5 whose 4-N, N, and N-Tori low-grade alkylamino phenyl group is a 4-N, N, and N-trimethyl aminophenyl group.

[Claim 7]A medicinal composition containing the cationic metalloporphyrin complex according to any one of claims 1 to 6.

[Claim 8]A HIDOROKI radical inducing agent containing the cationic metalloporphyrin complex according to any one of claims 1 to 6.

[Claim 9]An anticancer agent containing the cationic metalloporphyrin complex according to any one of claims 1 to 6.

[Claim 10]A medicinal composition containing a hydroxy radical inducing agent which can change

active oxygen in the living body into a hydroxy radical.

[Claim 11]The medicinal composition according to claim 10 whose medicinal composition is an anticancer agent.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[Field of the Invention]This invention relates to a new cationic metalloporphyrin complex and the medicinal composition containing it. In details, the medicinal composition of this invention is more useful as a SOD activator or an anticancer agent. A cancer cell is easy to be accumulated and the cationic metalloporphyrin complex of this invention is useful as a treating agent of various alternative tumors with few side effects.

[0002]

[Description of the Prior Art]In recent years, many chemical treatment methods by drugs came to be used as the method of cancer treatment. However, the actual condition is being unable to say that an operation is exerted also on a normal cell with a cancer cell since many drugs' do not have the singularity only to a cancer cell, for this reason intense side effects' are caused, and the chemotherapy is not necessarily working effectively. For example, although it is carried out for whether being clinical ** that cisplatin which is one of the anticancer agents used by clinical is effective in a uterine cancer, many side effects are also reported with validity. Although bioactive protein, such as interferon, TNF, and CSF, attracted attention from having cancer singularity, that the anticancer operation itself is not enough and since it cannot administer orally, it has not come to be widely used as an anticancer agent.

[0003]By the way, when the reactive oxygen species induced in the living body maintain the physiology activity of a living body, such as annihilating the different-species living thing which invaded in the living body, they are carrying out big work, but. The reactive oxygen species generated to coincidence in the living body more than needed may also destroy a self organization, and are said for generation of active oxygen more than needed to be not only harmful but a cause of aging. Although the anticancer agent which annihilates a cancer cell by changing oxygen in the living body into reactive oxygen species, and attacking a cancer cell nonspecific using the toxicity of these reactive oxygen species is also developed, it is nonspecific, and since a normal cell is also attacked, many side effects have been caused.

[0004]Although activating oxygen in the living body, making reactive oxygen species generate, and annihilating a cancer cell, when a certain kind of porphyrin system compound piles up a cancer cell etc. and irradiates this with a laser beam on the other hand is found out and it has been used as one of the anticancer agents with comparatively high singularity, A focus part must be made to irradiate with a laser beam, and when a focus part is in an inside, it has a fault, such as being ineffective.

[0005]

[Problem(s) to be Solved by the Invention]This invention provides the anticancer agent with few side effects based on the new mechanism of action which acts on a cancer cell specifically. It compares with a normal cell in a cancer cell, It is reported that the SOD activity is falling and the burst size of active oxygen is increasing (A. et). [V.Peskin, et al., FEBS Lett., 78, 41; (1977)

V.Leroyer,] al., Cancer Res., and 47-4771 (1987). This invention persons thought that the new anticancer mechanism which can attack a cancer cell specifically could be built, when the active oxygen which a cancer cell emits could be changed into the hydroxy radical ($-OH$) which has higher reactivity on that spot paying attention to this point.

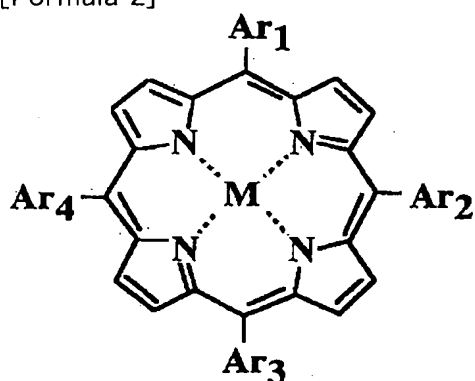
[0006]It found out that this invention persons have the SOD activity excellent in the new metalloporphyrin complex, and have the character specifically accumulated on a cancer cell, could react to active oxygen, and could make a hydroxy radical able to induce, and a cancer cell could be annihilated specifically. That is, this invention provides the new cationic metalloporphyrin complex in which side effects have little SOD activity with high safety. Since the new cationic metalloporphyrin complex of this invention accumulates on a cancer cell specifically, it is useful as an anticancer agent with few side effects, and this invention provides a new anticancer agent.

[0007]

[Means for Solving the Problem]This invention is the following general formula (1).

[0008]

[Formula 2]



(Among a formula, M shows the metal atom for forming a complex, Ar₁ it, and) Ar₂, Ar₃, and Ar₄ show carbocyclic or the heterocyclic aromatic group which may have a substituent independently, respectively, At least one piece is an aromatic group which has a cationic basis of Ar₁, Ar₂, Ar₃, and Ar₄. It is related with the cationic metalloporphyrin complex expressed.

[0009]This invention relates to a medicinal composition which consists of a carrier permitted on a cationic metalloporphyrin complex expressed with said general formula (1), and medicine manufacture. A medicinal composition of this invention is not only a hydroxy radical inducing agent, but it can use it as an anticancer agent. This invention relates to a medicinal composition which consists of a carrier permitted on a hydroxy radical inducing agent which can change active oxygen in the living body into a hydroxy radical on that spot, and medicine manufacture, especially an anticancer agent.

[0010]As for a cancer cell, it is known that antioxidant enzymes (SOD, catalase, etc.) are missing compared with a normal cell, for this reason a cancer cell has generated a superoxide radical so much compared with a normal cell. For example, if LLC-WRC-256 cell of a cancer cell, BRL-3 A cell of a normal cell, and the SOD activity of a natural antioxidant enzyme (Cu, Zn-SOD) are measured, it will become as it is shown in the next table 1.

[0011]

[Table 1]

表1 癌細胞と正常細胞におけるSOD活性の比較

細胞	IC ₅₀
	[$\times 10^3$ U/mg 蛋白質]
LLC-WRC-256細胞	8 ± 0.6
BRL-3A細胞	22 ± 1.2
Cu, Zn-SOD	760 ± 45

[0012] Thus, in a cancer cell, SOD activity is decreasing greatly compared with a normal cell. A cationic metalloporphyrin complex expressed with said general formula (1) of this invention has SOD activity, reacts to a superoxide radical specifically in the living body, and generates hydrogen peroxide. Since a cationic metalloporphyrin complex of this invention has metal at the center, generated hydrogen peroxide causes a harbor vice type reaction, and generates a toxic high hydroxy radical extremely, and it attacks only a cancer cell specifically.

[0013] On the other hand, in being the normal cell on which the antioxidant enzyme in the living body is functioning, Even if a superoxide radical hardly occurs but the cationic metalloporphyrin complex of this invention exists in a normal cell, it cannot react to a superoxide radical and a hydroxy radical cannot be generated. Therefore, since the cationic metalloporphyrin complex of this invention is a hydroxy radical inducing agent with the SOD activity which was alternative and was excellent, it is very useful as a high new anticancer agent of cancer cell singularity with few side effects.

[0014] The cationic metalloporphyrin complex of this invention has four aromatic groups in a porphin skeleton, and has a basis of cationicity [piece / at least one] of the four aromatic groups. independently, four aromatic groups may be carbocyclic things, or may be heterocyclic things, respectively -- monocyclic one -- even when -- it may be a thing of a polycyclic type. From the benzene ring, a naphthalene ring, etc., as an aromatic group, are a basis derived as a carbocyclic thing, and as a heterocyclic thing, It is a basis derived from the heterocycle of monocyclic [of 5 which has one piece or two nitrogen atoms or more, an oxygen atom, or a sulfur atom - 10 members], or a condensed ring type, for example, is a basis derived from a pyridine ring, a pyrimidine ring, an azole ring, etc. A phenyl group, 4-pyridyl group, etc. are mentioned as a desirable aromatic group.

[0015] These aromatic groups may have a substituent which does not have an adverse effect on SOD activity or an anticancer operation. As a substituent in an aromatic group, the carbon numbers 1-10, the straight chain of 1-6 or the low-grade alkyl group of the letter of branching, an amino group and the amino group preferably replaced by the above mentioned low-grade alkyl group, the lower alkoxy group that consists of the above mentioned low-grade alkyl group, etc. are mentioned.

[0016] As a cationic basis which an aromatic group has, although ammonium, a sulfonium group, etc. are mentioned, quaternary ammonium is preferred. Although it may have the cationic basis of this invention as a substituent of an aromatic group, the heteroatom of an aromatic group may be cation-ized. As an aromatic group which has a cationic basis, for example A 4-N, N, and N-trimethyl aminophenyl group, N-low-grade alkyl 4-pyridyl groups, such as 4-N,N,N-Tori low-grade alkylamino phenyl groups, such as a 4-N,N,N-triethyl aminophenyl group, an N-methyl-4-pyridyl group, and an N-ethyl-4-pyridyl group, etc. are mentioned.

[0017] Although at least one piece is an aromatic group which has a cationic basis of Ar₁ of said general formula (1) of this invention, Ar₂, Ar₃, and Ar₄, it has a desirable basis of cationicity [pieces / two or more] of these aromatic groups. As a thing of the porphyrin ring portion of a cationic metalloporphyrin complex expressed with said general formula (1) of this invention, Ar₁, Ar₂, Ar₃, and all the Ar₄ N-low-grade alkyl 4-pyridyl groups, such as an N-methyl-4-pyridyl group, Or the compound which is 4-N [, such as a 4-N, N, and N-trimethyl aminophenyl group,], N, and N-Tori

low-grade alkylamino phenyl group, Ar_1 , Ar_2 , and Ar_3 N-low-grade alkyl 4-pyridyl groups, such as an N-methyl-4-pyridyl group, Or it is 4-N [, such as a 4-N, N, and N-trimethyl aminophenyl group,], N, and N-Tori low-grade alkylamino phenyl group, The compound whose Ar_4 is a phenyl group, Ar_1 , and Ar_2 N-low-grade alkyl 4-pyridyl groups, such as an N-methyl-4-pyridyl group, or the compound Ar_3 and whose Ar_4 it is 4-N [, such as a 4-N, N, and N-trimethyl aminophenyl group], N, and N-Tori low-grade alkylamino phenyl group, and are phenyl groups and Ar_1 -- and, Ar_3 N-low-grade alkyl 4-pyridyl groups, such as an N-methyl-4-pyridyl group, Or the compound etc. Ar_2 and whose Ar_4 it is 4-N [, such as a 4-N, N, and N-trimethyl aminophenyl group,], N, and N-Tori low-grade alkylamino phenyl group, and are phenyl groups are mentioned.

[0018]As the central metal M in said general formula (1) of this invention, if SOD activity and an anticancer operation are shown, there will be no restriction in particular, but an iron atom, a copper atom, manganese atoms, etc. are mentioned preferably.

[0019]The cationic metalloporphyrin complex expressed with said general formula (1) of this invention can be manufactured in accordance with a publicly known method. For example, make pyrrole and aromatic aldehyde react and the Pori Phi Lynne ring portion is manufactured, This can be cation-ized with alkylating agents, such as halogenation low-grade alkyl and low-grade alkyl tosylate, and it can manufacture by the method of subsequently using as a metal complex using metal or metallic compounds, for example, a metal halogenide etc.

[0020]This invention persons considered the anticancer operation of various kinds of metalloporphyrin complexes using several sorts of cancer cells from which LLC-WRC and 256 cell of walker rat (Walker rat) cancer origin and SOD activity differ as a cancer cell. The procedure of this examination is typically shown in drawing 1. The compound which examined, and its abbreviation are as follows.

[0021]FeTM4PyP: The iron complex whose Ar_1 , Ar_2 , Ar_3 , and Ar_4 of a general formula (1) are an N-methyl-4-pyridyl group.

MnTM4PyP: The manganese complex whose Ar_1 , Ar_2 , Ar_3 , and Ar_4 of a general formula (1) are an N-methyl-4-pyridyl group.

CuTM4PyP: The copper complex whose Ar_1 , Ar_2 , Ar_3 , and Ar_4 of a general formula (1) are an N-methyl-4-pyridyl group.

FeTMAP : iron complex whose Ar_1 , Ar_2 , Ar_3 , and Ar_4 of a general formula (1) are a 4-N, N, and N-trimethyl aminophenyl group.

FeTSPP : iron complex whose Ar_1 , Ar_2 , Ar_3 , and Ar_4 of a general formula (1) are 4-sulfonate phenyl group.

[0022]Preparation of a porphyrin solution was performed using culture medium, and it adjusted so that porphyrin concentration might become [ml] in 10 and 50 or 100 microg /eventually.

Measurement added and cultivated the metalloporphyrin complex solution to the cancer cell developed on 12 hole plate, three days after addition, measured the rate of cell survival of each cancer cell with the trypan blue staining technique, and evaluated the anticancer effect.

[0023]The SOD activity of these metalloporphyrin complexes is correspondingly applied to T.Ohse, et al., Porphyrins, 6, and the method indicated to 137 (1997), The disproportionation kinetic constant (k_{cat}) of active oxygen (O_2^-) by a stopped-flow method estimated. k_{cat} made the HEPES/HEPES-Na buffer solution (pH 8.1) of the Pori Phi Lynne complex, and the DMSO solution of KO_2 react at 36 **, and was calculated from attenuation with an absorbance of 245 nm which is the absorption maximum wavelength of O_2^- . k_{cat} and IC_{50} in a metalloporphyrin complex and an antioxidant enzyme are shown in the next table 2. SOD activity is so high that the value of IC_{50} ** is so small

that the value of k_{cat} is large.

[0024]

[Table 2]

表2 各種ポルフィリン錯体の k_{cat} 値と IC_{50}

試験化合物	k_{cat} [$\times 10^{-6} M^{-1} s^{-1}$]	IC_{50} [$\mu g/ml$]
Cu, Zn-SOD	23.10	0.3
FeTM4PyP	22	0.8
FeTM4PyMPP	5.4	1.6
Fecis-DM4PyDPP	3.8	1.8
MnTM4PyP	22	0.7
FeTSPP	---	---

[0025]The intracellular SOD activity which a cancer cell has prepared the homogenate solution of the cell, and computed it with the chemiluminescence method using CLA. And the radical species ($-OH$) by which it was generated were measured according to ESR which used DMPO as a spin trap agent.

[0026]The extinction rate of the cancer cell using the cationic metalloporphyrin complex (100 microg/(ml)) of an invention by LLC-WRC and 256 cell and the antioxidant enzyme of bovine red cell origin (50 microg/(ml)) is shown in drawing 2. As a result of the rate evaluation of cell survival by the trypan blue staining technique of LLC-WRC and 256 cell, although the antioxidant enzyme has SOD activity, its anticancer effect is very low in itself. By metaled comparison, high FeTM4PyP of SOD activity and $-OH$ production ability showed the highest anticancer effect. It is thought that Mn complex which has the outstanding SOD activity depends on low $-OH$ production ability [that the effect was seldom accepted] of Mn complex. With Cu complex in which ***** and OH production ability are shown like Fe complex on the other hand, since SOD activity is low, it is thought that the anticancer effect became low compared with Fe complex.

[0027]The aforementioned result is shown in drawing 3 with the value of the disproportionation kinetic constant (k_{cat}) of active oxygen (O_2^-) used as the index of SOD activity. The numerical value in drawing 3 was $10^6 k_{cat} (M^{-1} s^{-1})$, and, incidentally the value of the antioxidant enzyme was $2300 \times 10^6 (M^{-1} s^{-1})$.

[0028]Next, this invention persons examined the accumulation ability to the cancer cell of a cationic metalloporphyrin complex. In order to consider the compatibility with a cell membrane and permeability which consist of a lipid membrane double layer, it examined about the different next compound of hydrophilic nature and hydrophobicity.

[0029]FeTM4PyP: The iron complex whose Ar_1 , Ar_2 , Ar_3 , and Ar_4 of a general formula (1) are an N-methyl-4-pyridyl group.

FeTM4PyMPPP: The iron complex whose Ar_4 , Ar_1 , Ar_2 , and Ar_3 of a general formula (1) are an N-methyl-4-pyridyl group, and is a phenyl group.

Fecis-DM4PyDPP: The iron complex Ar_3 and whose Ar_4 , Ar_1 and Ar_2 of a general formula (1) are an N-methyl-4-pyridyl group, and are phenyl groups.

Fetrans-DM4PyDPP: The iron complex Ar_2 and whose Ar_4 , Ar_1 and Ar_3 of a general formula (1) are an N-methyl-4-pyridyl group, and are phenyl groups.

[0030]The accumulation action to the cancer cell was checked by observing the red fluorescence of a porphyrin complex using a fluorescence microscope. Under [still a fixed quantity / porphyrin

complex / intracellular / atomic absorption analysis]. A result is shown in the next table 3.

[0031]

[Table 3]

表3 各種ポルフィリン錯体の癌細胞への集積能

試験化合物	取り込み量 [f m o l / 細胞]
F e T M 4 P y P	1 . 4 ± 0 . 1
F e T M 4 P y M P P	1 . 9 ± 0 . 2
F e D M 4 P y D P P	1 1 ± 0 . 8
M n T M 4 P y P	1 . 4 ± 0 . 2
F e T S P P	1 . 0 ± 0 . 1

[0032]The result checked that a hydrophobic, stronger porphyrin complex had higher accumulation ability. Especially as for FeDM4PyDPP, many complexes were incorporated into the cancer cell in the inside of a short time. By introducing a hydrophobic radical into a porphyrin complex, an interaction with a canal cell membrane becomes strong, and is considered that accumulation ability increased.

[0033]A temporal change of the anticancer effect about these compounds (100 microg/(ml)) was measured. This result is shown in drawing 4. The black triangle seal in drawing 4 shows FeTM4PyP, a black dot seal shows FeTM4PyMPP, and the black square seal shows FeDM4PyDPP, respectively. The used cancer cell is LLC-WRC and 256 cell of walker rat (Walker rat) cancer origin, and each compound was used for it by 100 microg/ml concentration. The vertical axis of drawing 4 shows the survival rate of the cancer cell, and the state where all the cancer cells survive is shown 100%.

[0034]FeDM4PyDPP which has two phenyl groups in a meso position annihilated almost all cancer cells within [in 24 hours] after addition. The anticancer effect decreased in order of FeDM4PyDPP>FeTM4PyP>FeTM4PyMPP.

[0035]The result of having measured the value of LD₅₀ (Median Lethal Dose: quantity of drugs required to annihilate a cell 50%) is shown in Table 4. The value of k_{cat} about the SOD activity measured by the method collectively mentioned above is shown in Table 4.

[0036]

[Table 4]

表4 各種ポルフィリン錯体の k_{cat} 値と LD₅₀

試験化合物	k _{cat} [× 1 0 ⁻⁶ M ⁻¹ s ⁻¹]	LD ₅₀ [μ M / m l]
F e T M 4 P y P	2 2	4 6
F e T M 4 P y M P P	5 . 4	1 5 0
F e D M 4 P y D P P	3 . 8	2 4
F e T M 4 P y P	2 2	--
F e T S P P	---	--

[0037]The disproportionation kinetic constant (k_{cat}) fell with reduction of the cationic substituent, and became the order of FeTM4PyP>FeTM4PyMPP>FeDM4PyDPP. However, FeDM4PyDPP made the anticancer operation which has SOD activity and in which high accumulation ability was excellent induce, though it is low. That is, when accumulation to a cancer cell increases remarkably by the hydrophobicity of FeDM4PyDPP, it is thought that the fall of the SOD activity by reduction of a cationic substituent was compensated, and the outstanding anticancer operation was shown. In

the anticancer effect that a cationic metalloporphyrin complex shows the accumulation ability from the above thing to a cancer cell, it turned out that it is an important factor.

[0038]Next, the operation on the normal cell of the compound of this invention was considered.

First, the growth experiment of the normal cell (BRL-3A) was conducted using mitomycin-C which is the compound and the publicly known anticancer antibiotic of this invention. A result is shown in drawing 5. The black dot seal in drawing 5 shows control, a black square seal shows the case where FeTM4PyP is added, a black triangle seal shows the case where MnTM4PyP is added, and a white round mark shows the case where mitomycin-C is added. When mitomycin-C which is the conventional anticancer agent is added, a cell number decreases with the passage of time, and it is set to 0 in about 70 hours. On the other hand, in the case of the compound of this invention, growth of the cell as well as [almost] control is performed. That is, it turns out that the compound of this invention hardly affects a normal cell.

[0039]FeTM4PyP which is a compound of this invention was added by various concentration in the culture medium of a normal cell (BRL-3 A cell) and a cancer cell (Walker256 cell), and the survival rate of each cell was measured. A result is shown in drawing 6. The white round mark in drawing 6 shows the survival rate of a cancer cell, and a black dot seal shows a normal cell. In a cancer cell, the concentration of FeTM4PyP receives that the survival rate falls to about 50%, and concentration falls [g / // 100 micro] to about 40% by ml further by ml in 50 microg /, In a normal cell, it turns out that the survival rate hardly falls even if it raises concentration, and the compound of this invention hardly affects it to a normal cell.

[0040]When the active principle of this invention generated a hydroxy radical specifically from the above result in the cancer cell which has much active oxygen, it became clear that it was what does the anticancer effect so. The anticancer agent by such a mechanism is based on the new idea by this invention persons, and this invention provides the cancer cell by a new mechanism with a specific new anticancer agent. The medicinal composition of this invention prescribes [taking orally or] for the patient continuously whether generally 1micro g-1 g is prescribed for the patient in several steps per day, although a medicine can be prescribed more for the patient parenteral and the effective dose is different by symptoms or a patient. The medicinal composition of this invention can be pharmaceutical-preparation-ized by a publicly known method, and can be suitably manufactured by the medication method or a patient.

[0041]

[Example]Next, although this invention is concretely explained based on an example, this invention is not limited to these examples.

[0042]Example 1 (composition of the Pori Flynn ring)

300 ml of propionic acid beforehand dried by Na_2SO_4 was put into the three neck flask, and under nitrogen, at 110 **, 7.5 ml (0.0707 is l) of benzaldehyde and 12.5 ml (0.1165 mol) of pyridin-4-aldehyde were put in, and it agitated and shaded. Subsequently, 12.5 ml (0.1863 mol) of pyrrole was dropped slowly. The solvent was distilled off after making it react for 2 hours. The ammonia solution neutralized and the solvent was distilled off. Reduced pressure drying was performed at 100 **. Washing filtration was carried out with methanol and reduced pressure drying of the residue was carried out. Separation refinement of the porphyrin (TPP, MPyTPP, DPyDPP, TPyMPP, TPyP) was carried out by the flash column (stationary phase: silica gel, 95% of developing solvent:methylene chloride 100%→ methanol 5% / methylene chloride). The cis object of DPyDPP and a trans object were not able to be separated this time. Isolation of each porphyrin was checked by NMR. Yield was about 2%.

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TECHNICAL FIELD

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PRIOR ART

[Description of the Prior Art]In recent years, many chemical treatment methods by drugs came to be used as the method of cancer treatment. However, the actual condition is being unable to say that an operation is exerted also on a normal cell with a cancer cell since many drugs' do not have the singularity only to a cancer cell, for this reason intense side effects' are caused, and the chemotherapy is not necessarily working effectively. For example, although it is carried out for whether being clinical ** that cisplatin which is one of the anticancer agents used by clinical is effective in a uterine cancer, many side effects are also reported with validity. Although bioactive protein, such as interferon, TNF, and CSF, attracted attention from having cancer singularity, that the anticancer operation itself is not enough and since it cannot administer orally, it has not come to be widely used as an anticancer agent.

[0003]By the way, when the reactive oxygen species induced in the living body maintain the physiology activity of a living body, such as annihilating the different-species living thing which invaded in the living body, they are carrying out big work, but. The reactive oxygen species generated to coincidence in the living body more than needed may also destroy a self organization, and are said for generation of active oxygen more than needed to be not only harmful but a cause of aging. Although the anticancer agent which annihilates a cancer cell by changing oxygen in the living body into reactive oxygen species, and attacking a cancer cell nonspecific using the toxicity of these reactive oxygen species is also developed, it is nonspecific, and since a normal cell is also attacked, many side effects have been caused.

[0004]Although activating oxygen in the living body, making reactive oxygen species generate, and annihilating a cancer cell, when a certain kind of porphyrin system compound piles up a cancer cell etc. and irradiates this with a laser beam on the other hand is found out and it has been used as one of the anticancer agents with comparatively high singularity, A focus part must be made to irradiate with a laser beam, and when a focus part is in an inside, it has a fault, such as being ineffective.

[Translation done.]

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EFFECT OF THE INVENTION

[Effect of the Invention]In this invention, the active oxygen which exists in a cancer cell so much is changed into a hydroxy radical ($-OH$).

Therefore, the new anticancer agent by the therapeutic method of cancer by the new mechanism of annihilating a cancer cell specifically, and this method is provided.

The anticancer agent of this invention has few side effects over a normal cell, and its curative effect is safely high. This invention provides the new metalloporphyrin complex which has growth control of a cancer cell, and an anticancer operation.

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TECHNICAL PROBLEM

[Problem(s) to be Solved by the Invention]This invention provides the anticancer agent with few side effects based on the new mechanism of action which acts on a cancer cell specifically. It compares with a normal cell in a cancer cell, It is reported that the SOD activity is falling and the burst size of active oxygen is increasing (A. et). [V.Peskin, et al., FEBS Lett., 78, 41; (1977) V.Leroyer,] al., Cancer Res., and 47-4771 (1987). This invention persons thought that the new anticancer mechanism which can attack a cancer cell specifically could be built, when the active oxygen which a cancer cell emits could be changed into the hydroxy radical (-OH) which has higher reactivity on that spot paying attention to this point.

[0006]It found out that this invention persons have the SOD activity excellent in the new metalloporphyrin complex, and have the character specifically accumulated on a cancer cell, could react to active oxygen, and could make a hydroxy radical able to induce, and a cancer cell could be annihilated specifically. That is, this invention provides the new cationic metalloporphyrin complex in which side effects have little SOD activity with high safety. Since the new cationic metalloporphyrin complex of this invention accumulates on a cancer cell specifically, it is useful as an anticancer agent with few side effects, and this invention provides a new anticancer agent.

[Translation done.]

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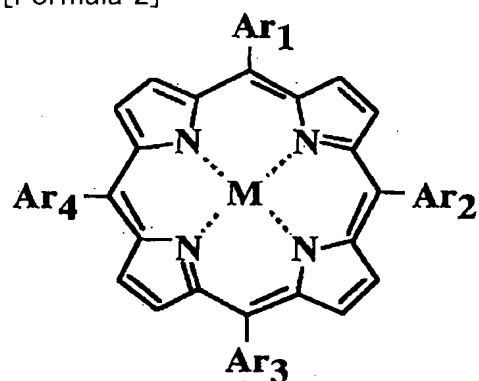
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MEANS

[Means for Solving the Problem]This invention is the following general formula (1).

[0008]

[Formula 2]



(Among a formula, M shows the metal atom for forming a complex, Ar₁ it, and) Ar₂, Ar₃, and Ar₄ show carbocyclic or the heterocyclic aromatic group which may have a substituent independently, respectively, At least one piece is an aromatic group which has a cationic basis of Ar₁, Ar₂, Ar₃, and Ar₄. It is related with the cationic metalloporphyrin complex expressed.

[0009]This invention relates to the medicinal composition which consists of a carrier permitted on the cationic metalloporphyrin complex expressed with said general formula (1), and medicine manufacture. The medicinal composition of this invention is not only a hydroxy radical inducing agent, but it can use it as an anticancer agent. This invention relates to the medicinal composition which consists of a carrier permitted on the hydroxy radical inducing agent which can change active oxygen in the living body into a hydroxy radical on that spot, and medicine manufacture, especially an anticancer agent.

[0010]As for a cancer cell, it is known that antioxidant enzymes (SOD, catalase, etc.) are missing compared with a normal cell, for this reason a cancer cell has generated a superoxide radical so much compared with a normal cell. For example, if LLC-WRC-256 cell of a cancer cell, BRL-3 A cell of a normal cell, and the SOD activity of a natural antioxidant enzyme (Cu, Zn-SOD) are measured, it will become as it is shown in the next table 1.

[0011]

[Table 1]

表1 癌細胞と正常細胞におけるSOD活性の比較

細胞	IC ₅₀ [×10 ³ U/mg蛋白質]
LLC-WRC-256細胞	8±0.6
BRL-3A細胞	22±1.2
Cu,Zn-SOD	760±45

[0012] Thus, in a cancer cell, SOD activity is decreasing greatly compared with a normal cell. A cationic metalloporphyrin complex expressed with said general formula (1) of this invention has SOD activity, reacts to a superoxide radical specifically in the living body, and generates hydrogen peroxide. Since a cationic metalloporphyrin complex of this invention has metal at the center, generated hydrogen peroxide causes a harbor vice type reaction, and generates a toxic high hydroxy radical extremely, and it attacks only a cancer cell specifically.

[0013] On the other hand, in being the normal cell on which the antioxidant enzyme in the living body is functioning, Even if a superoxide radical hardly occurs but the cationic metalloporphyrin complex of this invention exists in a normal cell, it cannot react to a superoxide radical and a hydroxy radical cannot be generated. Therefore, since the cationic metalloporphyrin complex of this invention is a hydroxy radical inducing agent with the SOD activity which was alternative and was excellent, it is very useful as a high new anticancer agent of cancer cell singularity with few side effects.

[0014] The cationic metalloporphyrin complex of this invention has four aromatic groups in a porphyrin skeleton, and has a basis of cationicity [piece / at least one] of the four aromatic groups. independently, four aromatic groups may be carbocyclic things, or may be heterocyclic things, respectively -- monocyclic one -- even when -- it may be a thing of a polycyclic type. From the benzene ring, a naphthalene ring, etc., as an aromatic group, are a basis derived as a carbocyclic thing, and as a heterocyclic thing, It is a basis derived from the heterocycle of monocyclic [of 5 which has one piece or two nitrogen atoms or more, an oxygen atom, or a sulfur atom - 10 members], or a condensed ring type, for example, is a basis derived from a pyridine ring, a pyrimidine ring, an azole ring, etc. A phenyl group, 4-pyridyl group, etc. are mentioned as a desirable aromatic group.

[0015] These aromatic groups may have a substituent which does not have an adverse effect on SOD activity or an anticancer operation. As a substituent in an aromatic group, the carbon numbers 1-10, the straight chain of 1-6 or the low-grade alkyl group of the letter of branching, an amino group and the amino group preferably replaced by the above mentioned low-grade alkyl group, the lower alkoxy group that consists of the above mentioned low-grade alkyl group, etc. are mentioned.

[0016] As a cationic basis which an aromatic group has, although ammonium, a sulfonium group, etc. are mentioned, quaternary ammonium is preferred. Although it may have the cationic basis of this invention as a substituent of an aromatic group, the heteroatom of an aromatic group may be cation-ized. As an aromatic group which has a cationic basis, for example A 4-N, N, and N-trimethyl aminophenyl group, N-low-grade alkyl 4-pyridyl groups, such as 4-N,N,N-Tori low-grade alkylamino phenyl groups, such as a 4-N,N,N-triethyl aminophenyl group, an N-methyl-4-pyridyl group, and an N-ethyl-4-pyridyl group, etc. are mentioned.

[0017] Although at least one piece is an aromatic group which has a cationic basis of Ar₁ of said general formula (1) of this invention, Ar₂, Ar₃, and Ar₄, it has a desirable basis of cationicity

[pieces / two or more] of these aromatic groups. As a thing of the porphyrin ring portion of a cationic metalloporphyrin complex expressed with said general formula (1) of this invention, Ar₁, Ar₂, Ar₃, and all the Ar₄ N-low-grade alkyl 4-pyridyl groups, such as an N-methyl-4-pyridyl group, Or the compound which is 4-N [, such as a 4-N, N, and N-trimethyl aminophenyl group,], N, and N-Tori

low-grade alkylamino phenyl group, Ar_1 , Ar_2 , and Ar_3 N-low-grade alkyl 4-pyridyl groups, such as an N-methyl-4-pyridyl group, Or it is 4-N [, such as a 4-N, N, and N-trimethyl aminophenyl group,], N, and N-Tori low-grade alkylamino phenyl group, The compound whose Ar_4 is a phenyl group, Ar_1 , and Ar_2 N-low-grade alkyl 4-pyridyl groups, such as an N-methyl-4-pyridyl group, or the compound Ar_3 and whose Ar_4 it is 4-N [, such as a 4-N, N, and N-trimethyl aminophenyl group], N, and N-Tori low-grade alkylamino phenyl group, and are phenyl groups and Ar_1 -- and, Ar_3 N-low-grade alkyl 4-pyridyl groups, such as an N-methyl-4-pyridyl group, Or the compound etc. Ar_2 and whose Ar_4 it is 4-N [, such as a 4-N, N, and N-trimethyl aminophenyl group,], N, and N-Tori low-grade alkylamino phenyl group, and are phenyl groups are mentioned.

[0018]As the central metal M in said general formula (1) of this invention, if SOD activity and an anticancer operation are shown, there will be no restriction in particular, but an iron atom, a copper atom, manganese atoms, etc. are mentioned preferably.

[0019]The cationic metalloporphyrin complex expressed with said general formula (1) of this invention can be manufactured in accordance with a publicly known method. For example, make pyrrole and aromatic aldehyde react and the Pori Phi Lynne ring portion is manufactured, This can be cation-ized with alkylating agents, such as halogenation low-grade alkyl and low-grade alkyl tosylate, and it can manufacture by the method of subsequently using as a metal complex using metal or metallic compounds, for example, a metal halogenide etc.

[0020]This invention persons considered the anticancer operation of various kinds of metalloporphyrin complexes using several sorts of cancer cells from which LLC-WRC and 256 cell of walker rat (Walker rat) cancer origin and SOD activity differ as a cancer cell. The procedure of this examination is typically shown in drawing 1. The compound which examined, and its abbreviation are as follows.

[0021]FeTM4PyP: The iron complex whose Ar_1 , Ar_2 , Ar_3 , and Ar_4 of a general formula (1) are an N-methyl-4-pyridyl group.

MnTM4PyP: The manganese complex whose Ar_1 , Ar_2 , Ar_3 , and Ar_4 of a general formula (1) are an N-methyl-4-pyridyl group.

CuTM4PyP: The copper complex whose Ar_1 , Ar_2 , Ar_3 , and Ar_4 of a general formula (1) are an N-methyl-4-pyridyl group.

FeTMAP : iron complex whose Ar_1 , Ar_2 , Ar_3 , and Ar_4 of a general formula (1) are a 4-N, N, and N-trimethyl aminophenyl group.

FeTSPP : iron complex whose Ar_1 , Ar_2 , Ar_3 , and Ar_4 of a general formula (1) are 4-sulfonate phenyl group.

[0022]Preparation of a porphyrin solution was performed using culture medium, and it adjusted so that porphyrin concentration might become [ml] in 10 and 50 or 100 microg /eventually.

Measurement added and cultivated the metalloporphyrin complex solution to the cancer cell developed on 12 hole plate, three days after addition, measured the rate of cell survival of each cancer cell with the trypan blue staining technique, and evaluated the anticancer effect.

[0023]The SOD activity of these metalloporphyrin complexes is correspondingly applied to T.Ohse, et al., Porphyrins, 6, and the method indicated to 137 (1997), The disproportionation kinetic constant (k_{cat}) of active oxygen (O_2^-) by a stopped-flow method estimated. k_{cat} made the HEPES/HEPES-Na buffer solution (pH 8.1) of the Pori Phi Lynne complex, and the DMSO solution of KO_2 react at 36 **, and was calculated from attenuation with an absorbance of 245 nm which is the absorption maximum wavelength of O_2^- . k_{cat} and IC_{50} in a metalloporphyrin complex and an antioxidant enzyme are shown in the next table 2. SOD activity is so high that the value of IC_{50} ** is so small

that the value of k_{cat} is large.

[0024]

[Table 2]

表2 各種ポルフィリン錯体の k_{cat} 値と IC_{50}

試験化合物	k_{cat} [$\times 10^{-6} \text{M}^{-1} \text{s}^{-1}$]	IC_{50} [$\mu\text{g}/\text{ml}$]
Cu, Zn-SOD	2310	0.3
FeTM4PyP	22	0.8
FeTM4PyMPP	5.4	1.6
Fecis-DM4PyDPP	3.8	1.8
MnTM4PyP	22	0.7
FeTSP	---	---

[0025]The intracellular SOD activity which a cancer cell has prepared the homogenate solution of the cell, and computed it with the chemiluminescence method using CLA. And the radical species ($\cdot\text{OH}$) by which it was generated were measured according to ESR which used DMPO as a spin trap agent.

[0026]The extinction rate of the cancer cell using the cationic metalloporphyrin complex (100 microg/(ml)) of an invention by LLC-WRC and 256 cell and the antioxidant enzyme of bovine red cell origin (50 microg/(ml)) is shown in drawing 2. As a result of the rate evaluation of cell survival by the trypan blue staining technique of LLC-WRC and 256 cell, although the antioxidant enzyme has SOD activity, its anticancer effect is very low in itself. By metaled comparison, high FeTM4PyP of SOD activity and $\cdot\text{OH}$ production ability showed the highest anticancer effect. It is thought that Mn complex which has the outstanding SOD activity depends on low $\cdot\text{OH}$ production ability [that the effect was seldom accepted] of Mn complex. With Cu complex in which ***** and $\cdot\text{OH}$ production ability are shown like Fe complex on the other hand, since SOD activity is low, it is thought that the anticancer effect became low compared with Fe complex.

[0027]The aforementioned result is shown in drawing 3 with the value of the disproportionation kinetic constant (k_{cat}) of active oxygen ($\text{O}_2\cdot^-$) used as the index of SOD activity. The numerical value in drawing 3 was $10^6 k_{\text{cat}}$ ($\text{M}^{-1}\text{s}^{-1}$), and, incidentally the value of the antioxidant enzyme was 2300×10^6 ($\text{M}^{-1}\text{s}^{-1}$).

[0028]Next, this invention persons examined the accumulation ability to the cancer cell of a cationic metalloporphyrin complex. In order to consider the compatibility with a cell membrane and permeability which consist of a lipid membrane double layer, it examined about the different next compound of hydrophilic nature and hydrophobicity.

[0029]FeTM4PyP: The iron complex whose Ar_1 , Ar_2 , Ar_3 , and Ar_4 of a general formula (1) are an N-methyl-4-pyridyl group.

FeTM4PyMPPP: The iron complex whose Ar_4 , Ar_1 , Ar_2 , and Ar_3 of a general formula (1) are an N-methyl-4-pyridyl group, and is a phenyl group.

Fecis-DM4PyDPP: The iron complex Ar_3 and whose Ar_4 , Ar_1 and Ar_2 of a general formula (1) are an N-methyl-4-pyridyl group, and are phenyl groups.

Fetrans-DM4PyDPP: The iron complex Ar_2 and whose Ar_4 , Ar_1 and Ar_3 of a general formula (1) are an N-methyl-4-pyridyl group, and are phenyl groups.

[0030]The accumulation action to the cancer cell was checked by observing the red fluorescence of a porphyrin complex using a fluorescence microscope. Under [still a fixed quantity / porphyrin

complex / intracellular / atomic absorption analysis]. A result is shown in the next table 3.

[0031]

[Table 3]

表3 各種ポルフィリン錯体の癌細胞への集積能

試験化合物	取り込み量 [f m o l / 細胞]
F e T M 4 P y P	1 . 4 ± 0 . 1
F e T M 4 P y M P P	1 . 9 ± 0 . 2
F e D M 4 P y D P P	1 1 ± 0 . 8
M n T M 4 P y P	1 . 4 ± 0 . 2
F e T S P P	1 . 0 ± 0 . 1

[0032]The result checked that a hydrophobic, stronger porphyrin complex had higher accumulation ability. Especially as for FeDM4PyDPP, many complexes were incorporated into the cancer cell in the inside of a short time. By introducing a hydrophobic radical into a porphyrin complex, an interaction with a canal cell membrane becomes strong, and is considered that accumulation ability increased.

[0033]A temporal change of the anticancer effect about these compounds (100 microg/(ml)) was measured. This result is shown in drawing 4. The black triangle seal in drawing 4 shows FeTM4PyP, a black dot seal shows FeTM4PyMPP, and the black square seal shows FeDM4PyDPP, respectively. The used cancer cell is LLC-WRC and 256 cell of walker rat (Walker rat) cancer origin, and each compound was used for it by 100 microg/ml concentration. The vertical axis of drawing 4 shows the survival rate of the cancer cell, and the state where all the cancer cells survive is shown 100%.

[0034]FeDM4PyDPP which has two phenyl groups in a meso position annihilated almost all cancer cells within [in 24 hours] after addition. The anticancer effect decreased in order of FeDM4PyDPP>FeTM4 PyP>FeTM4PyMPP.

[0035]The result of having measured the value of LD₅₀ (Median Lethal Dose: quantity of drugs required to annihilate a cell 50%) is shown in Table 4. The value of k_{cat} about the SOD activity measured by the method collectively mentioned above is shown in Table 4.

[0036]

[Table 4]

表4 各種ポルフィリン錯体のk_{cat}値とLD₅₀

試験化合物	k _{cat} [× 1 0 ⁻⁶ M ⁻¹ s ⁻¹]	LD ₅₀ [μ M / m l]
F e T M 4 P y P	2 2	4 6
F e T M 4 P y M P P	5 . 4	1 5 0
F e D M 4 P y D P P	3 . 8	2 4
F e T M 4 P y P	2 2	--
F e T S P P	---	--

[0037]The disproportionation kinetic constant (k_{cat}) fell with reduction of the cationic substituent, and became the order of FeTM4 PyP>FeTM4 PyMPP>FeDM4PyDPP. However, FeDM4PyDPP made the anticancer operation which has SOD activity and in which high accumulation ability was excellent induce, though it is low. That is, when accumulation to a cancer cell increases remarkably by the hydrophobicity of FeDM4PyDPP, it is thought that the fall of the SOD activity by reduction of a cationic substituent was compensated, and the outstanding anticancer operation was shown. In

the anticancer effect that a cationic metalloporphyrin complex shows the accumulation ability from the above thing to a cancer cell, it turned out that it is an important factor.

[0038]Next, the operation on the normal cell of the compound of this invention was considered. First, the growth experiment of the normal cell (BRL-3A) was conducted using mitomycin-C which is the compound and the publicly known anticancer antibiotic of this invention. A result is shown in drawing 5. The black dot seal in drawing 5 shows control, a black square seal shows the case where FeTM4PyP is added, a black triangle seal shows the case where MnTM4PyP is added, and a white round mark shows the case where mitomycin-C is added. When mitomycin-C which is the conventional anticancer agent is added, a cell number decreases with the passage of time, and it is set to 0 in about 70 hours. On the other hand, in the case of the compound of this invention, growth of the cell as well as [almost] control is performed. That is, it turns out that the compound of this invention hardly affects a normal cell.

[0039]FeTM4PyP which is a compound of this invention was added by various concentration in the culture medium of a normal cell (BRL-3 A cell) and a cancer cell (Walker256 cell), and the survival rate of each cell was measured. A result is shown in drawing 6. The white round mark in drawing 6 shows the survival rate of a cancer cell, and a black dot seal shows a normal cell. In a cancer cell, the concentration of FeTM4PyP receives that the survival rate falls to about 50%, and concentration falls [g / // 100 micro] to about 40% by ml further by ml in 50 microg /, In a normal cell, it turns out that the survival rate hardly falls even if it raises concentration, and the compound of this invention hardly affects it to a normal cell.

[0040]When the active principle of this invention generated a hydroxy radical specifically from the above result in the cancer cell which has much active oxygen, it became clear that it was what does the anticancer effect so. The anticancer agent by such a mechanism is based on the new idea by this invention persons, and this invention provides the cancer cell by a new mechanism with a specific new anticancer agent. The medicinal composition of this invention prescribes [taking orally or] for the patient continuously whether generally 1micro g-1 g is prescribed for the patient in several steps per day, although a medicine can be prescribed more for the patient parenteral and the effective dose is different by symptoms or a patient. The medicinal composition of this invention can be pharmaceutical-preparation-ized by a publicly known method, and can be suitably manufactured by the medication method or a patient.

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EXAMPLE

[Example]Next, although this invention is concretely explained based on an example, this invention is not limited to these examples.

[0042]Example 1 (composition of the Pori Flynn ring)

300 ml of propionic acid beforehand dried by Na_2SO_4 was put into the three neck flask, and under nitrogen, at 110 **, 7.5 ml (0.0707 is l) of benzaldehyde and 12.5 ml (0.1165 mol) of pyridin-4-aldehyde were put in, and it agitated and shaded. Subsequently, 12.5 ml (0.1863 mol) of pyrrole was dropped slowly. The solvent was distilled off after making it react for 2 hours. The ammonia solution neutralized and the solvent was distilled off. Reduced pressure drying was performed at 100 **. Washing filtration was carried out with methanol and reduced pressure drying of the residue was carried out. Separation refinement of the porphyrin (TPP, MPyTPP, DPyDPP, TPyMPP, TPyP) was carried out by the flash column (stationary phase: silica gel, 95% of developing solvent:methylene chloride 100%→ methanol 5% / methylene chloride). The cis object of DPyDPP and a trans object were not able to be separated this time. Isolation of each porphyrin was checked by NMR. Yield was about 2%.

[0043]Example 2 (methylation of porphyrin (class[the / fourth]-izing of N atom of the pyridyl group of a meso position))

Ethanol 10% (3 ml) / chloroform 90% (27 ml) of a solvent is put into a three-neck flask, Each porphyrin (in the case [In the case / In the case of MPyTPP / of 0.077 g (1.3×10^{-4} Mol) and DPyDPP] of 0.235 g (3.8×10^{-4} mol) and TPyMPP 0.251g (3.9×10^{-4} mol)) was added. P-toluenesulfonic-acid methyl of a number of N atom of about 4 time molar quantity to methylate (to MPyTPP, 0.120 ml (6.4×10^{-4} mol)) To DPyDPP, 0.88 ml (4.7×10^{-3} mol) was added to 0.57 ml (3.1×10^{-3} mol) and TPyMPP, and it shaded under 35 ** and nitrogen, and was made to react all night. After distilling off a solvent, reduced pressure drying was performed and MMPyTPP (what methylated MPyTPP), DPyDPP (what methylated DPyPPP), and TMPyMPP (what methylated TPyMPP) were obtained.

[0044]TMPyMPP was melted in methanol of the amount of marks after this, was reprecipitated in diethylether, and collected after-filtration residue. Since it was dramatically refractory in the direction of DMPyPPP, this process was not performed. Reduced pressure drying was fully performed. Since it was insoluble to water, MMPyTPP was not able to be used here. The check of each methylation was performed by NMR (refer to drawing 7, drawing 8, and drawing 9). Yield was about 45%.

[0045]

^1H -NMR [270 MHz, $\text{DMSO}-d_6$]: delta DMPyDPP 9.48 (8H, 2, 6-pyridyl)

9.20 (8H, pyrrole- beta)

9.00 (8H, 3, 5-pyridyl)

4.73 (12H, N-methyl)

-3.10 (2H, internal pyrrole)

TMPyMPP 9.71-7.30(46H)

4.96 (9H, N-methyl)

-2.74 (2H, internal pyrrole)

TMPyP 9.44-7.07(40H)

4.71 (6H, N-methyl)

-2.90 (2H, internal pyrrole)

[0046]Example 3 (metal (Fe) introduction to porphyrin)

Put 150 ml of succinic acid buffer solution (pH4.05) into a three neck flask, and under 80 °C and nitrogen, Each porphyrin (in DMPyDPP, 0.257 g (2.7×10^{-4} mol)) In TMPyMPP, 0.207 g (1.8×10^{-4} mol) is added, It is the about 10 time molar quantity (0.425 g (2.1×10^{-3} mol) to DMPyDPP) of porphyrin about ferric chloride (FeCl_3) 4 hydrate. 0.382g (1.9×10^{-3} mol) is put in to TMPyMPP, and a UV-Vis spectrum is measured for every hour, and it was made to react until it was able to check metal introduction from the shift of a peak, and an absorbance. The iron separated in the column (stationary phase: ion-exchange resin HP20, 10% of developing solvent:water → water / methanol 90%) was separated after distilling off a solvent, reduced pressure drying after distilling off was performed for the solvent, and FeDMPyDPP and FeTMPyMPP were obtained. Yield was about 70%.

[0047]Example 4 (cell culture)

After thawing the cell which carried out cryopreservation with 37 °C warm water, it moves to a centrifugation tube, Except for DMSO which centrifuges by 1000 rotations for 10 minutes, throws away a supernatant fluid, and is used in the case of cryopreservation, culture medium (it expresses MEM hereafter.) was put in, and after suspension, it moved to the culture flask and cultivated within the incubator (under 37 °C and carbon dioxide). After it exchanged MEM every two to three days and a cell fully increased, trypsinization was carried out, the cell was stripped from the flask, and it moved to the centrifugation tube, and for 10 minutes, the supernatant fluid after centrifugal separation was thrown away by 1000 rotations, MEM was added, it moved to some flasks after suspension (passage), and the cell was proliferated. Under the present circumstances, LLC-WRC-256 cell cultured Eagle's essential medium (it expresses E-MEM hereafter.) and BRL-3 A cell using Ham's F-12K (it expresses F-12K hereafter.).

[0048]Example 5 (carcinostatic activity evaluation)

The cell which carried out subculture was stripped from the culture flask by trypsinization, and it moved to the centrifugation tube, and for 10 minutes, after centrifugal separation and a supernatant fluid were thrown away by 1000 rotations, MEM was added, and every 1 ml per hole was added to the dish of 12 holes after suspension. Since it will cultivate by an incubator for one day and the cell was implanted, each porphyrin complex prepared so that final concentration might become in ml and ten to 100 microg /and mitomycin-C, or carboplatin was added, and it put into the incubator. Evaluation was taken out from the incubator after fixed time lapse (0.5 to 120 hours after), and it carried out by dyeing an extinction cell with a trypan blue solution. MEM was attracted after fixed time lapse, the trypan blue solution was put in after washing by PBS, and it put into the incubator for 15 minutes. It washed by after-suction PBS, PBS was put in again, and a photograph was taken by observing under a culture microscope (OLYMPUS done type culture microscope IMT[of a handstand]-2) (OLYMPUS microphotograph automatic exposure photographing instrument modelPM-10-A). The number of the extinction cell dyed by the trypan blue from the obtained photograph and the viable cells which have not dyed was measured, and the rate of cell survival was computed. The above procedure is typically shown in drawing 1.

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DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]

[Drawing 1]Drawing 1 shows a measuring method for the survival rate of a cancer cell typically.

[Drawing 2]Drawing 2 graph-izes the extinction rate to the cancer cell of the cationic metalloporphyrin complex of this invention, and an antioxidant enzyme, and shows it.

[Drawing 3]Drawing 3 graph-izes the extinction rate to the cancer cell of various metalloporphyrin complexes and an antioxidant enzyme, and shows k_{cat} value of the SOD activity.

[Drawing 4]Drawing 4 graph-izes the survival rate over the cancer cell of the cationic metalloporphyrin complex of this invention, and shows it. The black triangle seal in drawing 4 shows FeTM4PyP, a black dot seal shows FeTM4PyMPP, and the black square seal shows FeDM4PyDPP, respectively.

[Drawing 5]Drawing 5 graph-izes an operation on the normal cell in the cationic metalloporphyrin complex and the publicly known anticancer agent of this invention, and shows it.

[Drawing 6]Drawing 6 graph-izes influence of the cancer cell and normal cell by a cationic metalloporphyrin complex on this invention, and shows it.

[Drawing 7]Drawing 7 shows the NMR chart of the compound before metalization of the cationic metalloporphyrin complex (DMPyDPP) of this invention.

[Drawing 8]Drawing 8 shows the NMR chart of the compound before metalization of the cationic metalloporphyrin complex (TMPyMPP) of this invention.

[Drawing 9]Drawing 9 shows the NMR chart of the compound before metalization of the cationic metalloporphyrin complex (TMPyP) of this invention.

[Translation done.]

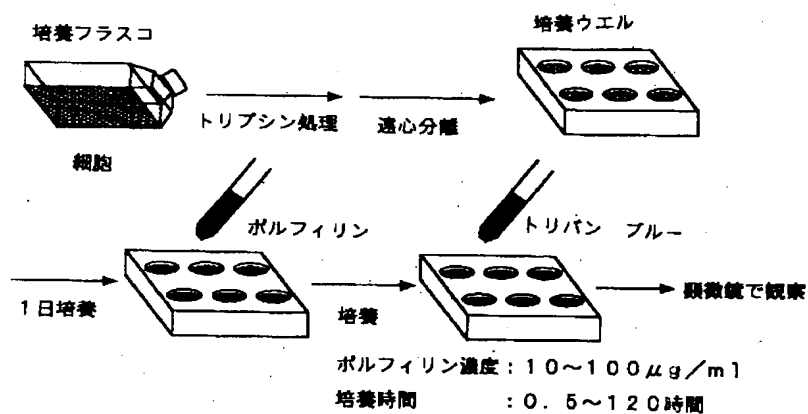
* NOTICES *

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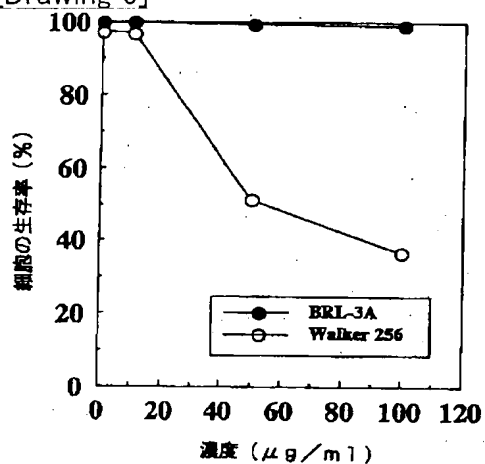
- 1.This document has been translated by computer. So the translation may not reflect the original precisely.
- 2.**** shows the word which can not be translated.
- 3.In the drawings, any words are not translated.

DRAWINGS

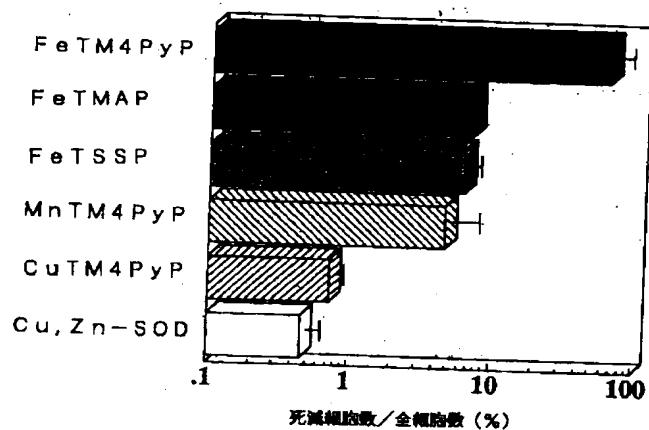
[Drawing 1]



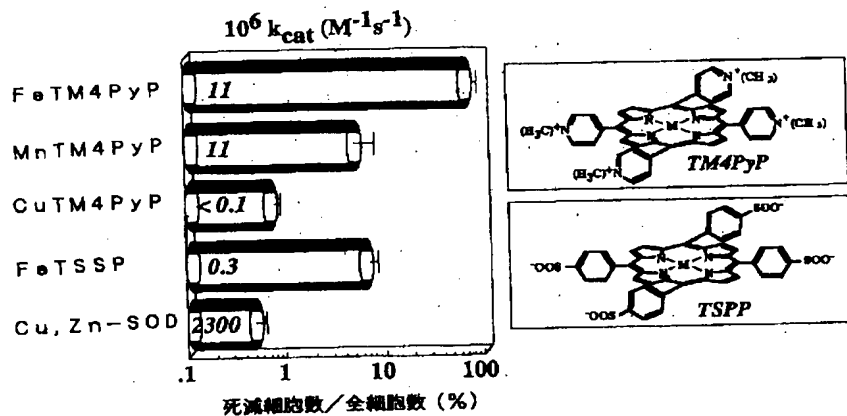
[Drawing 6]



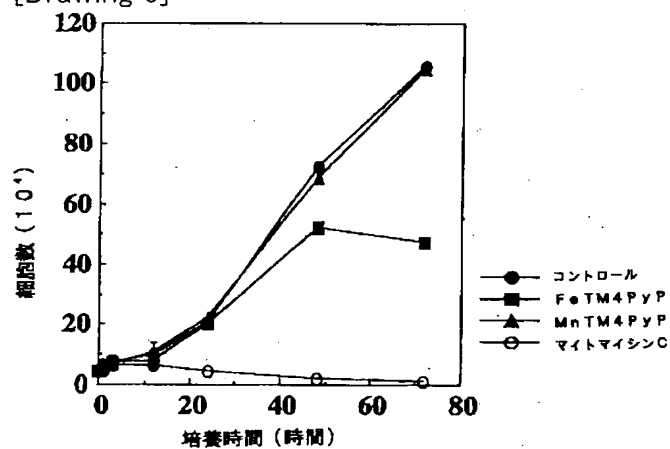
[Drawing 2]



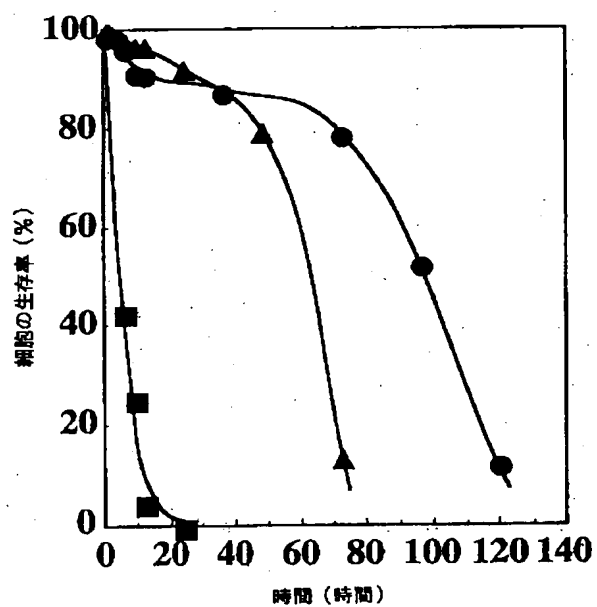
[Drawing 3]



[Drawing 5]

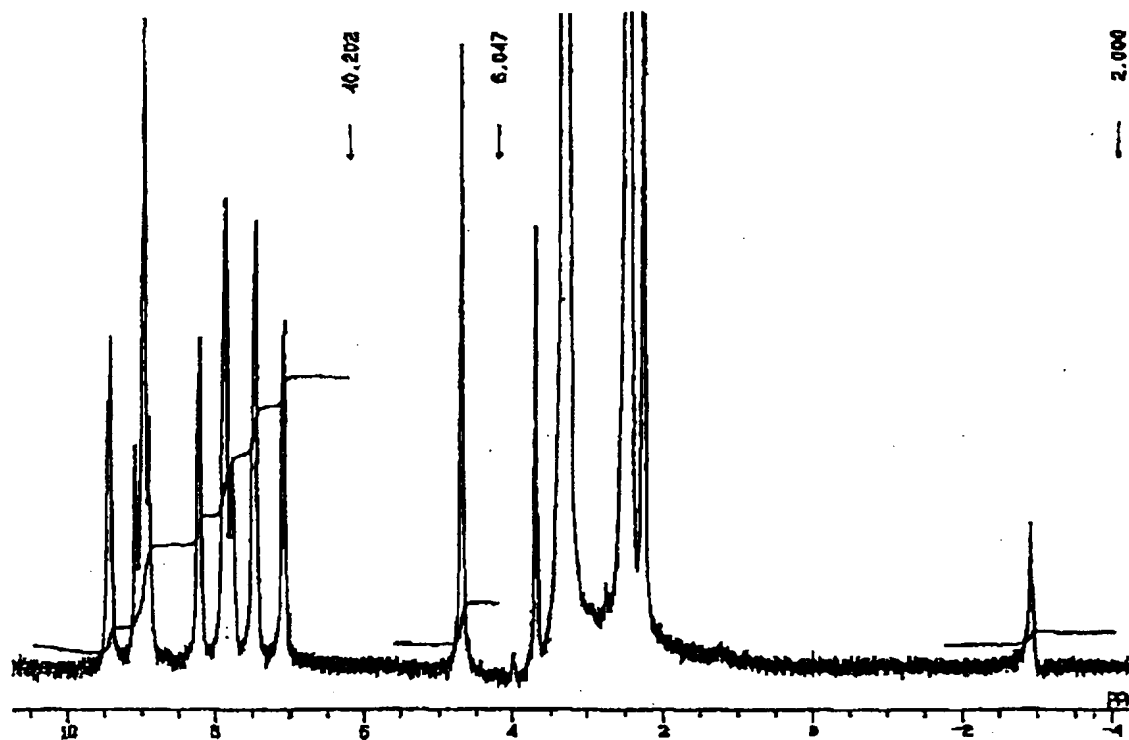


[Drawing 4]

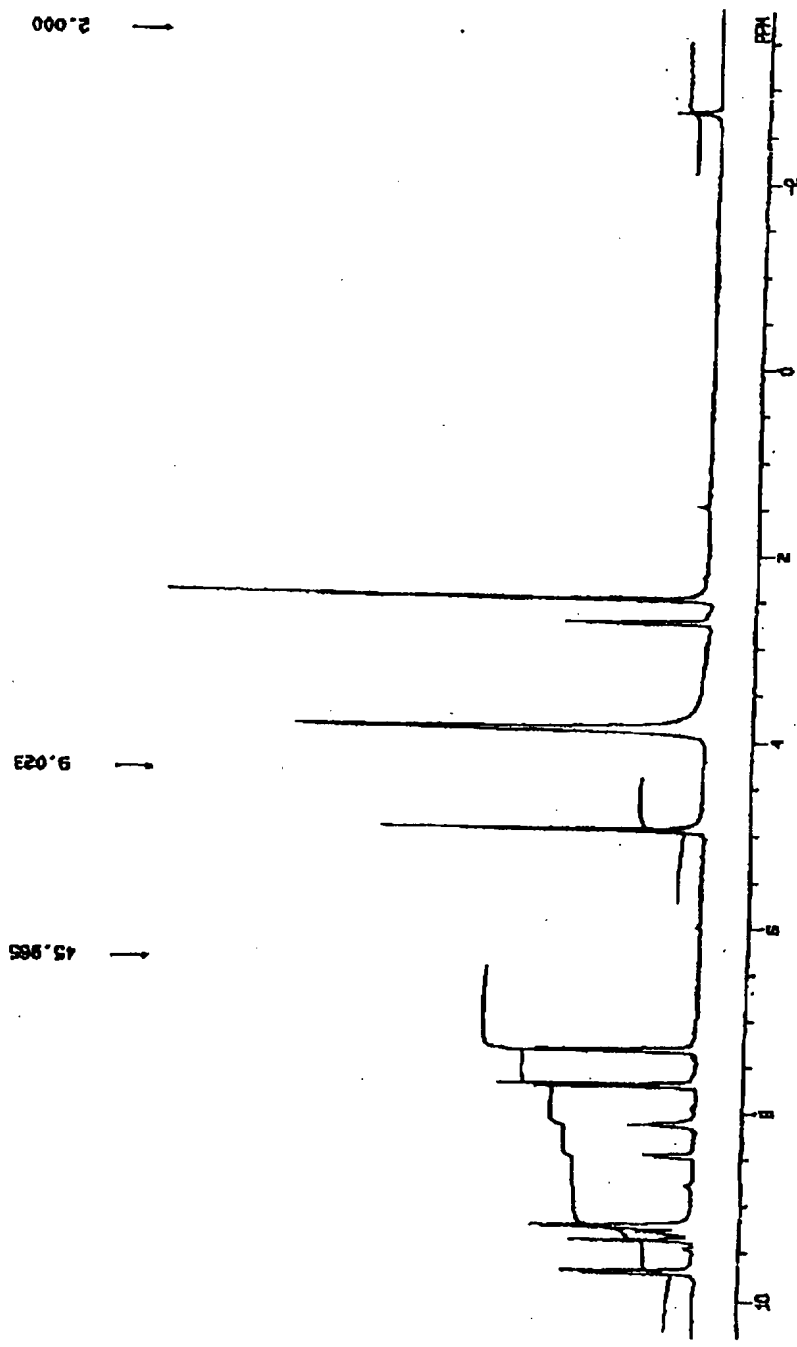


▲ : FeMPy₄P
 ● : FeMPy₃P₁P
 ■ : FeMPy₂P₂P

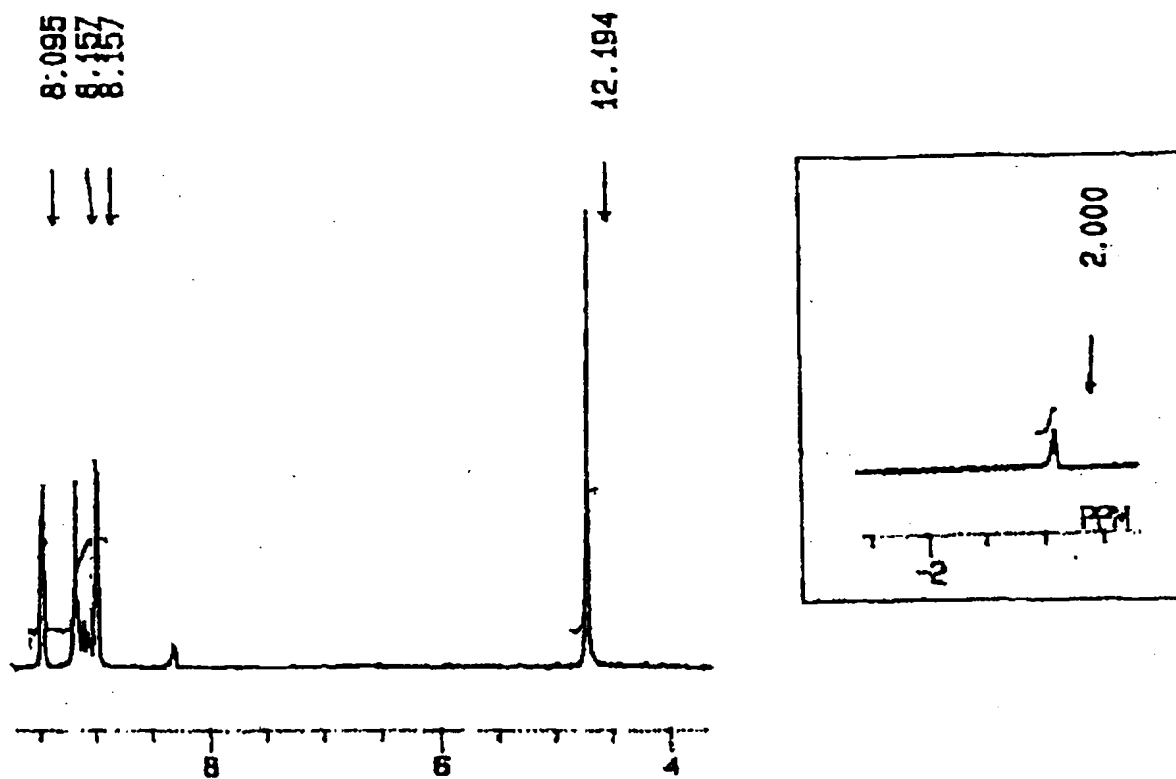
[Drawing 7]



[Drawing 8]



[Drawing 9]



[Translation done.]